

United States Patent Application

of

**Peter S. Dragovich, Thomas J. Prins, and Ru Zhou**

for

**ANTIPICORNAVIRAL COMPOUNDS, COMPOSITIONS CONTAINING  
THEM, AND METHODS FOR THEIR USE**

- 1 -

TITLE

**ANTIPICORNAVIRAL COMPOUNDS, COMPOSITIONS CONTAINING  
THEM, AND METHODS FOR THEIR USE**

**RELATED APPLICATIONS**

This application is a division of U.S. Patent Application No. 09/947,381, filed on September 7, 2001, which was a division of U.S. Patent Application No. 09/421,560, filed on October 20, 1999, now U.S. Patent No. 6,331,554, which is a division of U.S. Patent Application No. 08/991,282, filed December 16, 1997, now U.S. Patent No. 6,030,371, which is a continuation-in-part of U.S. Patent Application No. 08/825,331, filed March 28, 1997, now abandoned.

Additionally, this application relates to U.S. Patent Application No. 08/850,398, filed May 2, 1997, now U.S. Patent No. 5,856,530; to U.S. Patent Application No. 08/991,739, filed December 16, 1997, now U.S. Patent No. 5,962,487; and to U.S. Provisional Application No. 60/046,204, filed May 12, 1997. Each of these applications are entirely incorporated herein by reference.

## BACKGROUND OF THE INVENTION

**[0001]** The invention pertains to the discovery and use of new compounds that inhibit the enzymatic activity of picornaviral 3C proteases, specifically rhinovirus proteases (RVPs), as well as retard viral growth in cell culture.

**[0002]** The picornaviruses are a family of tiny non-enveloped positive stranded RNA containing viruses that infect humans and other animals. These viruses include the human rhinoviruses, human polioviruses, human coxsackieviruses, human echoviruses, human and bovine enteroviruses, encephalomyocarditis viruses, menigovirus, foot and mouth viruses, hepatitis A virus and others. The human rhinoviruses are a major cause of the common cold. To date, there are no effective therapies to cure the common cold, only treatments that relieve the symptoms.

**[0003]** Picornaviral infections may be treated by inhibiting the proteolytic 3C enzymes. These enzymes are required for the natural maturation of the picornaviruses. They are responsible for the autocatalytic cleavage of the genomic, large polyprotein into the essential viral proteins. Members of the 3C protease family are cysteine proteases, where the sulfhydryl group most often cleaves the glutamine-glycine amide bond. Inhibition of 3C proteases is believed to block proteolytic cleavage of the polyprotein, which in turn can retard the maturation and replication of the viruses by interfering with viral particle production. Therefore, inhibiting the processing of this cysteine protease with selective, small molecules that are specifically recognized should represent an important and useful approach to treat and cure viral infections of this nature and, in particular, the common cold.

## SUMMARY OF THE INVENTION

**[0004]** The present invention is directed to compounds that function as picornaviral 3C protease inhibitors, particularly those that have antiviral activity. It is further directed to the use of such 3C protease inhibitors. The Inventors demonstrate that the compounds of the present invention bind to rhinovirus 3C proteases and preferably have antiviral cell culture activity. The enzymatic





Q' is -C(R<sub>10</sub>)(R<sub>11</sub>)- or -O-, X is -CH- or -CF-, and Y<sub>1</sub> is -CH-, -CF-, or -C(alkyl)-, where R<sub>10</sub> and R<sub>11</sub> independently are H, a halogen, or an alkyl group, or, together with the carbon atom to which they are attached, form a cycloalkyl group or a heterocycloalkyl group,

or X is -CH<sub>2</sub>-, -CF<sub>2</sub>-, -CHF-, or -S-, and Y<sub>1</sub> is -O-, -S-, -NR<sub>12</sub>-, -C(R<sub>13</sub>)(R<sub>14</sub>)-, -C(O)-, -C(S)-, or -C(CR<sub>13</sub>R<sub>14</sub>)-,

wherein R<sub>12</sub> is H or alkyl, and R<sub>13</sub> and R<sub>14</sub> independently are H, F, or an alkyl group, or, together with the atoms to which they are bonded, form a cycloalkyl group or a heterocycloalkyl group;

A<sub>1</sub> is C, CH, CF, S, P, Se, N, NR<sub>15</sub>, S(O), Se(O), P-OR<sub>15</sub>, or P-NR<sub>15</sub>R<sub>16</sub>,

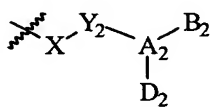
wherein R<sub>15</sub> and R<sub>16</sub> independently are an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group, or, together with the atom to which they are bonded, form a heterocycloalkyl group;

D<sub>1</sub> is a moiety with a lone pair of electrons capable of forming a hydrogen bond; and

B<sub>1</sub> is H, F, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -OR<sub>17</sub>, -SR<sub>17</sub>, -NR<sub>17</sub>R<sub>18</sub>, -NR<sub>19</sub>NR<sub>17</sub>R<sub>18</sub>, or -NR<sub>17</sub>OR<sub>18</sub>,

wherein R<sub>17</sub>, R<sub>18</sub>, and R<sub>19</sub> independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group;

and with the provisos that when  $D_1$  is the moiety  $\equiv N$  with a lone pair of electrons capable of forming a hydrogen bond,  $B_1$  does not exist; and when  $A_1$  is an  $sp^3$  carbon,  $B_1$  is not  $-NR_{17}R_{18}$  when  $D_1$  is the moiety  $-NR_{25}R_{26}$  with a lone pair of electrons capable of forming a hydrogen bond, wherein  $R_{25}$  and  $R_{26}$  are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group;  
and wherein  $D_1-A_1-B_1$  optionally forms a nitro group where  $A_1$  is N;  
and further wherein, when  $R_2$  or  $R_5$  is



X is  $=CH$  or  $=CF$  and  $Y_2$  is  $=C$ ,  $=CH$ , or  $=CF$ ,

or X and  $Y_2$  together with  $Q'$  form a three-membered ring in which  $Q'$  is  $-C(R_{10})(R_{11})-$  or  $-O-$ , X is  $-CH-$  or  $-CF-$ , and  $Y_2$  is  $-CH-$ ,  $-CF-$ , or  $-C(alkyl)-$ , where  $R_{10}$  and  $R_{11}$  independently are H, a halogen, or an alkyl group, or, together with the carbon atom to which they are attached, form a cycloalkyl group or a heterocycloalkyl group,

or X is  $-CH_2-$ ,  $-CF_2-$ ,  $-CHF-$ , or  $-S-$ , and  $Y_2$  is  $-O-$ ,  $-S-$ ,  $-N(R'_{12})-$ ,  $-C(O)-$ ,  $-C(R'_{13})(R'_{14})-$ ,  $-C(S)-$ , or  $-C(CR'_{13}R'_{14})-$ ,

wherein  $R'_{12}$  is H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group,  $-OR'_{13}$ ,  $-NR'_{13}R'_{14}$ ,  $-C(O)-R'_{13}$ ,  $-SO_2R'_{13}$ , or  $-C(S)R'_{13}$ , and  $R'_{13}$  and  $R'_{14}$ , independently are H, F, or an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or

a heteroaryl group, or, together with the atom to which they are attached, form a cycloalkyl group or a heterocycloalkyl group;

A<sub>2</sub> is C, CH, CF, S, P, Se, N, NR<sub>15</sub>, S(O), Se(O), P-OR<sub>15</sub>, or P-NR<sub>15</sub>R<sub>16</sub>, wherein R<sub>15</sub> and R<sub>16</sub> independently are an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group or, together with the atom to which they are bonded, form a heterocycloalkyl group;

D<sub>2</sub> is a moiety with a lone pair of electrons capable of forming a hydrogen bond; and

B<sub>2</sub> is H, F, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -OR<sub>17</sub>, -SR<sub>17</sub>, -NR<sub>17</sub>R<sub>18</sub>, -NR<sub>19</sub>NR<sub>17</sub>R<sub>18</sub>, or -NR<sub>17</sub>OR<sub>18</sub>,

wherein R<sub>17</sub>, R<sub>18</sub>, and R<sub>19</sub> independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group;

and further wherein any combination of Y<sub>2</sub>, A<sub>2</sub>, B<sub>2</sub>, and D<sub>2</sub> forms a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group;

R<sub>3</sub> and R<sub>6</sub> are independently H, F, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -C(O)R<sub>17</sub>, -OR<sub>17</sub>, -SR<sub>17</sub>, -NR<sub>17</sub>R<sub>18</sub>, -NR<sub>19</sub>NR<sub>17</sub>R<sub>18</sub>, or -NR<sub>17</sub>OR<sub>18</sub>,

wherein R<sub>17</sub>, R<sub>18</sub>, and R<sub>19</sub> independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an

acyl group;

or, R<sub>3</sub> and R<sub>6</sub>, together with the carbon atom to which they are attached, form a cycloalkyl group or a heterocycloalkyl group;

R<sub>7</sub> is H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -OR<sub>17</sub>, -SR<sub>17</sub>, -NR<sub>17</sub>R<sub>18</sub>, -NR<sub>19</sub>NR<sub>17</sub>R<sub>18</sub>, or -NR<sub>17</sub>OR<sub>18</sub>,

wherein R<sub>17</sub>, R<sub>18</sub>, and R<sub>19</sub> independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group;

or R<sub>7</sub>, together with R<sub>3</sub> or R<sub>6</sub> and the atoms to which they are attached, form a heterocycloalkyl group;

R<sub>20</sub> is H, OH, or any suitable organic moiety; and

Z and Z<sub>1</sub> are independently H, F, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -C(O)R<sub>21</sub>, -CO<sub>2</sub>R<sub>21</sub>, -CN, -C(O)NR<sub>21</sub>R<sub>22</sub>, -C(O)NR<sub>21</sub>OR<sub>22</sub>, -C(S)R<sub>21</sub>, -C(S)NR<sub>21</sub>R<sub>22</sub>, -NO<sub>2</sub>, -SOR<sub>21</sub>, -SO<sub>2</sub>R<sub>21</sub>, -SO<sub>2</sub>NR<sub>21</sub>R<sub>22</sub>, -SO(NR<sub>21</sub>)(OR<sub>22</sub>), -SONR<sub>21</sub>, -SO<sub>3</sub>R<sub>21</sub>, -PO(OR<sub>21</sub>)<sub>2</sub>, -PO(R<sub>21</sub>)(R<sub>22</sub>), -PO(NR<sub>21</sub>R<sub>22</sub>)(OR<sub>23</sub>), -PO(NR<sub>21</sub>R<sub>22</sub>)(NR<sub>23</sub>R<sub>24</sub>), -C(O)NR<sub>21</sub>NR<sub>22</sub>R<sub>23</sub>, or -C(S)NR<sub>21</sub>NR<sub>22</sub>R<sub>23</sub>,

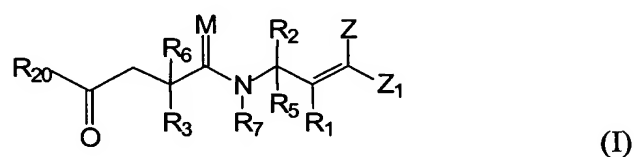
wherein R<sub>21</sub>, R<sub>22</sub>, R<sub>23</sub>, and R<sub>24</sub> are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, an acyl group, or a thioacyl group, or wherein any two of R<sub>21</sub>, R<sub>22</sub>, R<sub>23</sub>, and R<sub>24</sub>, together with the atom(s) to which they are bonded, form a heterocycloalkyl group;

or Z<sub>1</sub>, as defined above, together with R<sub>1</sub>, as defined above, and the atoms to which Z<sub>1</sub> and R<sub>1</sub> are bonded, form a cycloalkyl or heterocycloalkyl

group,  
or Z and Z<sub>1</sub>, both as defined above, together with the atoms to which they  
are bonded, form a cycloalkyl or heterocycloalkyl group;  
and pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates  
thereof,  
and wherein these compounds, pharmaceutically acceptable prodrugs, salts, active  
metabolites, and solvates preferably have antipicornaviral activity with an EC<sub>50</sub>  
less than or equal to 10 μM in the HI-HeLa cell culture assay, and more preferably  
antirhinoviral activity with an EC<sub>50</sub> less than or equal to 10 μM in the HI-HeLa cell  
culture assay and/or antioxsachieviral activity with an EC<sub>50</sub> less than or equal to  
10 μM in the HI-HeLa cell culture assay.

## DETAILED DESCRIPTION OF THE INVENTION

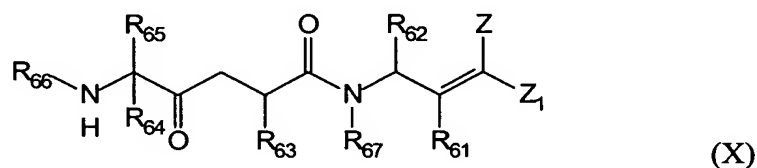
[0006] The present invention relates to compounds of the formula I:



wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>20</sub>, M, Z, and Z<sub>1</sub> are as defined above, and to the  
pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates  
thereof, where these compounds, pharmaceutically acceptable prodrugs, salts,  
active metabolites, and solvates preferably have antipicornaviral activity with an  
EC<sub>50</sub> less than or equal to 10 μM in the HI-HeLa cell culture assay, and more  
preferably antirhinoviral activity with an EC<sub>50</sub> less than or equal to 10 μM in the

HI-HeLa cell culture assay and/or anticomplexing activity with an  $EC_{50}$  less than or equal to 10  $\mu M$  in the HI-HeLa cell culture assay.

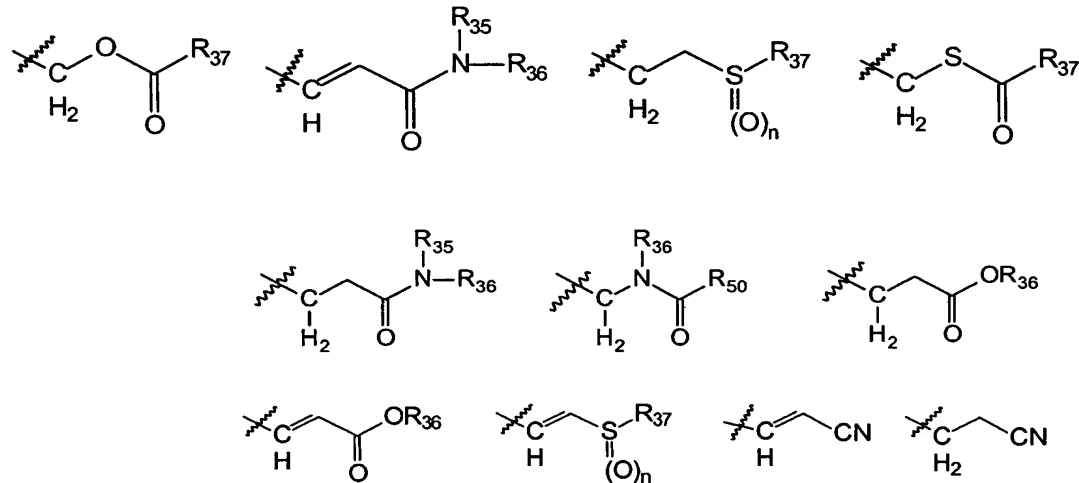
[0007] The present invention preferably relates to compounds of the formula X:



wherein

$R_{61}$  is H, F or an alkyl group;

$R_{62}$  is selected from one of the following moieties:



wherein

$R_{35}$  is H, an alkyl group, an aryl group,  $-OR_{38}$ , or  $-NR_{38}R_{39}$ ,

wherein  $R_{38}$  and  $R_{39}$  independently are H, an alkyl group, a

cycloalkyl group, a heterocycloalkyl

group, an aryl group, a heteroaryl group,

or an acyl group; and

R<sub>36</sub> is H or an alkyl group,

or R<sub>35</sub> and R<sub>36</sub>, together with the nitrogen atom to which they are attached, form a heterocycloalkyl group or a heteroaryl group;

R<sub>37</sub> is an alkyl group, an aryl group, or -NR<sub>38</sub>R<sub>39</sub>, wherein R<sub>38</sub> and R<sub>39</sub> are as defined above;

R<sub>50</sub> is H, an alkyl group, an aryl group, -OR<sub>38</sub>, -SR<sub>39</sub>, -NR<sub>38</sub>R<sub>39</sub>, -NR<sub>40</sub>NR<sub>38</sub>R<sub>39</sub>, or

-NR<sub>38</sub>OR<sub>39</sub>, or R<sub>50</sub> and R<sub>36</sub>, together with the atoms to which they are attached, form a heterocycloalkyl group;

wherein R<sub>38</sub> and R<sub>39</sub> are as defined above and R<sub>40</sub> is H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group; and

n is 0, 1, or 2;

R<sub>63</sub> is H or an alkyl group;

R<sub>64</sub> is H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group;

R<sub>65</sub> is H or an alkyl group;

R<sub>66</sub> is H, an acyl group, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a sulfonyl group, or a heteroaryl group;

R<sub>67</sub> is H or an alkyl group;

and

Z and Z<sub>1</sub> are independently H, F, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, -C(O)R<sub>21</sub>, -CO<sub>2</sub>R<sub>21</sub>, -

CN, -C(O)NR<sub>21</sub>R<sub>22</sub>,  
-C(O)NR<sub>21</sub>OR<sub>22</sub>, -C(S)R<sub>21</sub>, -C(S)NR<sub>21</sub>R<sub>22</sub>, -NO<sub>2</sub>, -SOR<sub>21</sub>, -SO<sub>2</sub>R<sub>21</sub>, -SO<sub>2</sub>NR<sub>21</sub>R<sub>22</sub>,  
-SO(NR<sub>21</sub>)(OR<sub>22</sub>), -SONR<sub>21</sub>, -SO<sub>3</sub>R<sub>21</sub>, -PO(OR<sub>21</sub>)<sub>2</sub>, -PO(R<sub>21</sub>)(R<sub>22</sub>), -  
PO(NR<sub>21</sub>R<sub>22</sub>)(OR<sub>23</sub>), -PO(NR<sub>21</sub>R<sub>22</sub>)(NR<sub>23</sub>R<sub>24</sub>), -C(O)NR<sub>21</sub>NR<sub>22</sub>R<sub>23</sub>, or -  
C(S)NR<sub>21</sub>NR<sub>22</sub>R<sub>23</sub>,

wherein R<sub>21</sub>, R<sub>22</sub>, R<sub>23</sub>, and R<sub>24</sub> are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, an acyl group, or a thioacyl group, or wherein any two of R<sub>21</sub>, R<sub>22</sub>, R<sub>23</sub>, and R<sub>24</sub>, together with the atom(s) to which they are bonded, form a heterocycloalkyl group,

or Z and Z<sub>1</sub>, both as defined above, together with the atoms to which they are attached, form a heterocycloalkyl group;

and pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates thereof.

**[0008]** As used in the present application, the following definitions apply:

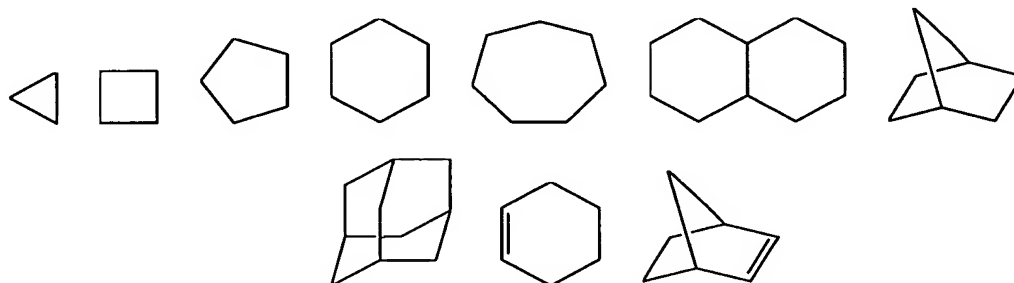
**[0009]** An "alkyl group" is intended to mean a straight or branched chain monovalent radical of saturated and/or unsaturated carbon atoms and hydrogen atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, ethenyl, pentenyl, butenyl, propenyl, ethynyl, butynyl, propynyl, pentynyl, hexynyl, and the like, which may be unsubstituted (i.e., containing only carbon and hydrogen) or substituted by one or more suitable substituents as defined below.

**[0010]** A "cycloalkyl group" is intended to mean a non-aromatic, monovalent monocyclic, bicyclic, or tricyclic radical containing 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 carbon ring atoms, each of which may be saturated or unsaturated, and which may be unsubstituted or substituted by one or more suitable substituents as defined below, and to which may be fused one or more heterocycloalkyl groups,

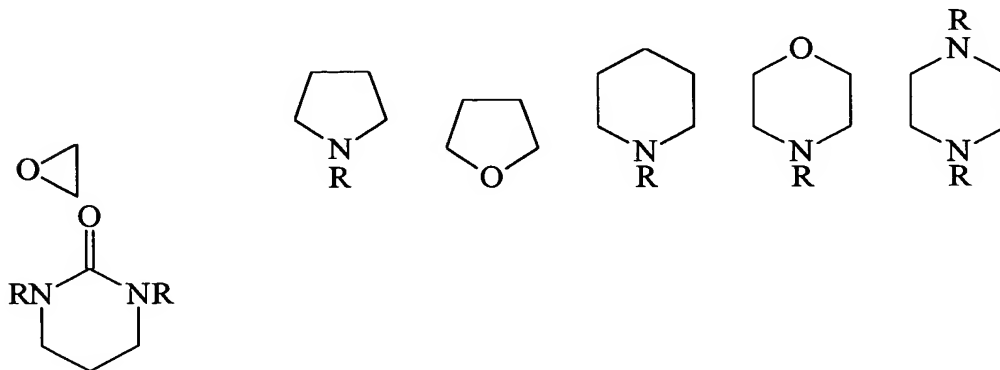


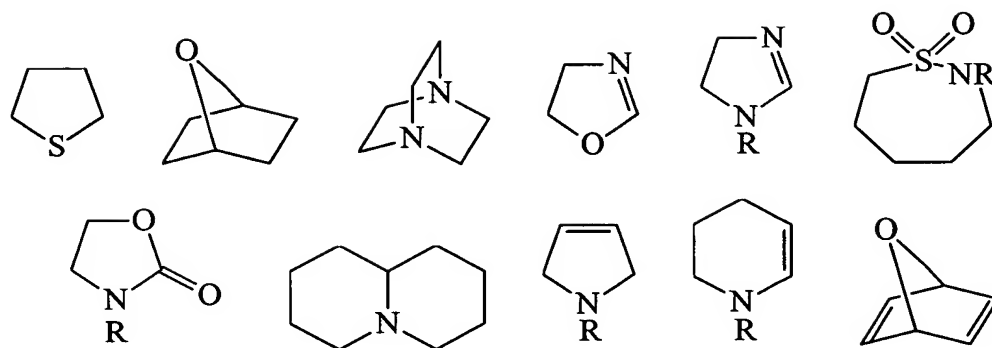
aryl groups, or heteroaryl groups, which themselves may be unsubstituted or substituted by one or more suitable substituents.

**[0011]** Illustrative examples of cycloalkyl groups include, but are not limited to, the following moieties:

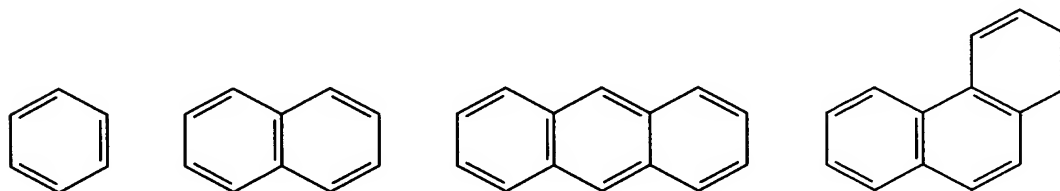


**[0012]** A "heterocycloalkyl group" is intended to mean a non-aromatic, monovalent monocyclic, bicyclic, or tricyclic radical, which is saturated or unsaturated, containing 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 ring atoms, and which includes 1, 2, 3, 4, or 5 heteroatoms selected from nitrogen, oxygen, and sulfur, wherein the radical is unsubstituted or substituted by one or more suitable substituents as defined below, and to which may be fused one or more cycloalkyl groups, aryl groups, or heteroaryl groups, which themselves may be unsubstituted or substituted by one or more suitable substituents. Illustrative examples of heterocycloalkyl groups include, but are not limited to the following moieties:

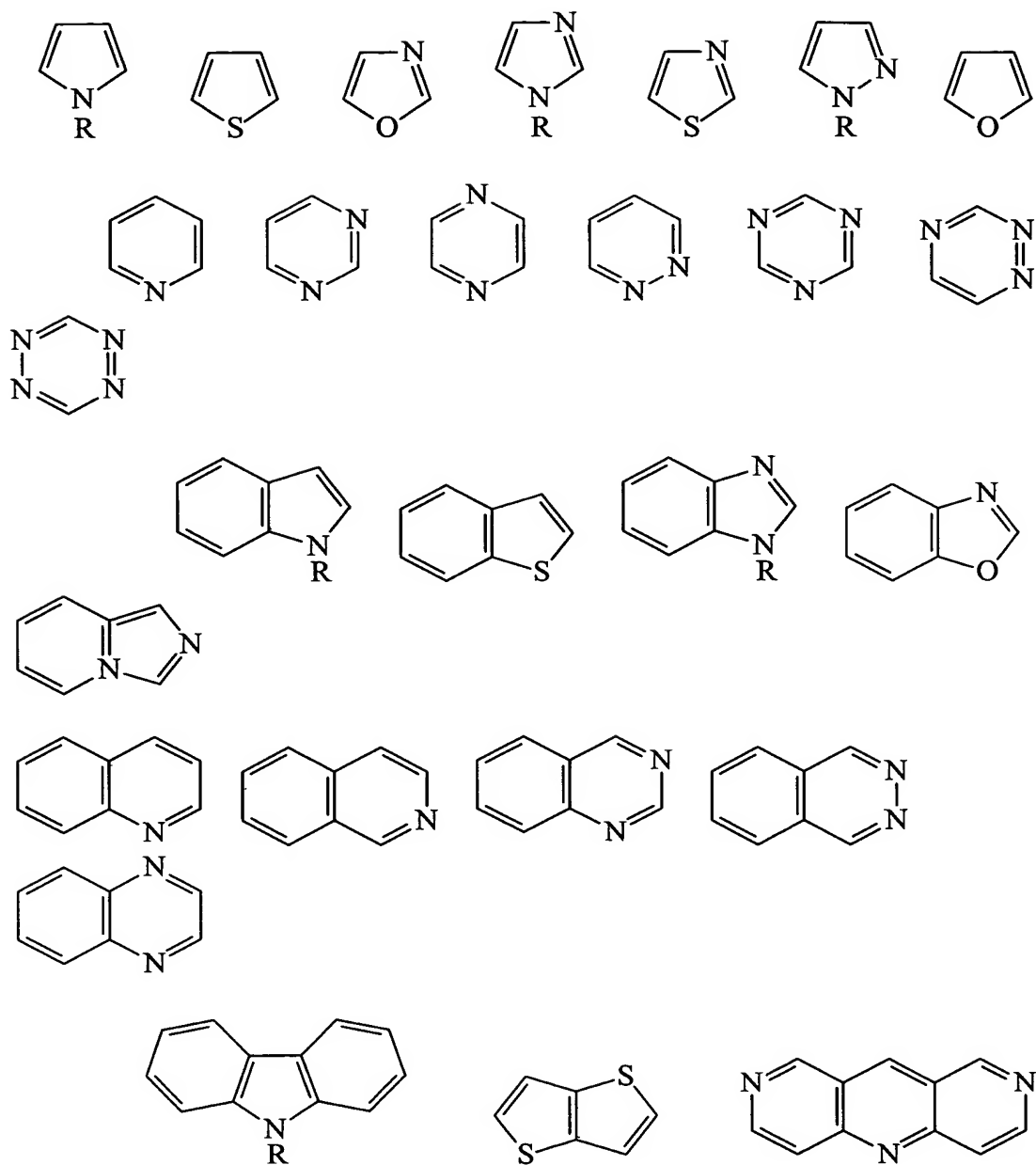




**[0013]** An "aryl group" is intended to mean an aromatic, monovalent monocyclic, bicyclic, or tricyclic radical containing 6, 10, 14, 18 carbon ring atoms, which may be unsubstituted or substituted by one or more suitable substituents as defined below, and to which may be fused one or more cycloalkyl groups, heterocycloalkyl groups, or heteroaryl groups, which themselves may be unsubstituted or substituted by one or more suitable substituents. Illustrative examples of aryl groups include, but are not limited to, the following moieties:



**[0014]** A "heteroaryl group" is intended to mean an aromatic monovalent monocyclic, bicyclic, or tricyclic radical containing 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 ring atoms, including 1, 2, 3, 4, or 5 heteroatoms selected from nitrogen, oxygen, and sulfur, which may be unsubstituted or substituted by one or more suitable substituents as defined below, and to which may be fused one or more cycloalkyl groups, heterocycloalkyl groups, or aryl groups, which themselves may be unsubstituted or substituted by one or more suitable substituents. Illustrative examples of heteroaryl groups include, but are not limited to, the following moieties:



**[0015]** An "acyl group" is intended to mean a -C(O)-R radical, wherein R is any suitable substituent as defined below.

**[0016]** A "thioacyl group" is intended to mean a -C(S)-R radical, wherein R is any suitable substituent as defined below.

**[0017]** A "sulfonyl group" is intended to mean a -SO<sub>2</sub>R radical, wherein R is any suitable substituent as defined below.

**[0018]** The term "suitable substituent" is intended to mean any of the substituents recognizable, such as by routine testing, to those skilled in the art as not adversely affecting the inhibitory activity of the inventive compounds. Illustrative examples of suitable substituents include, but are not limited to, hydroxy groups, oxo groups, alkyl groups, acyl groups, sulfonyl groups, mercapto groups, alkylthio groups, alkoxy groups, cycloalkyl groups, heterocycloalkyl groups, aryl groups, heteroaryl groups, carboxy groups, amino groups, alkylamino groups, dialkylamino groups, carbamoyl groups, aryloxy groups, heteroaryloxy groups, arylthio groups, heteroarylthio groups, and the like.

**[0019]** The term "suitable organic moiety" is intended to mean any organic moiety recognizable, such as by routine testing, to those skilled in the art as not adversely affecting the inhibitory activity of the inventive compounds. Illustrative examples of suitable organic moieties include, but are not limited to, hydroxy groups, alkyl groups, oxo groups, cycloalkyl groups, heterocycloalkyl groups, aryl groups, heteroaryl groups, acyl groups, sulfonyl groups, mercapto groups, alkylthio groups, alkoxy groups, carboxy groups, amino groups, alkylamino groups, dialkylamino groups, carbamoyl groups, arylthio groups, heteroarylthio groups, and the like.

**[0020]** A "hydroxy group" is intended to mean the radical -OH.

**[0021]** An "amino group" is intended to mean the radical -NH<sub>2</sub>.

**[0022]** An "alkylamino group" is intended to mean the radical -NHR where R is an alkyl group as defined above.

**[0023]** A "dialkylamino group" is intended to mean the radical -NR<sub>a</sub>R<sub>b</sub> where R<sub>a</sub> and R<sub>b</sub> are each independently an alkyl group as defined above.

**[0024]** An "alkoxy group" is intended to mean the radical -OR where R is an alkyl group as defined above, for example, methoxy, ethoxy, propoxy, and the like.

**[0025]** An "alkoxycarbonyl group" is intended to mean the radical -C(O)OR where R is an alkyl group as defined above.

[0026] An "alkylsulfonyl group" is intended to mean the radical  $\text{-SO}_2\text{R}$  where R is an alkyl group as defined above.

[0027] An "alkylaminocarbonyl group" is intended to mean the radical  $\text{-C(O)NHR}$  where R is an alkyl group as defined above.

[0028] A "dialkylaminocarbonyl group" is intended to mean the radical  $\text{-C(O)NR}_a\text{R}_b$  where  $\text{R}_a$  and  $\text{R}_b$  are each independently an alkyl group as defined above.

[0029] A "mercapto group" is intended to mean the radical  $\text{-SH}$ .

[0030] An "alkylthio group" is intended to mean the radical  $\text{-SR}$  where R is an alkyl group as defined above.

[0031] A "carboxy group" is intended to mean the radical  $\text{-C(O)OH}$ .

[0032] A "carbamoyl group" is intended to mean the radical  $\text{-C(O)NH}_2$ .

[0033] An "aryloxy group" is intended to mean the radical  $\text{-OR}_c$  where  $\text{R}_c$  is an aryl group as defined above.

[0034] A "heteroaryloxy group" is intended to mean the radical  $\text{-OR}_d$  where  $\text{R}_d$  is a heteroaryl group as defined above.

[0035] An "arylthio group" is intended to mean the radical  $\text{-SR}_c$  where  $\text{R}_c$  is an aryl group as defined above.

[0036] A "heteroarylthio group" is intended to mean the radical  $\text{-SR}_d$  where  $\text{R}_d$  is a heteroaryl group as defined above.

[0037] A "pharmaceutically acceptable prodrug" is intended to mean a compound that may be converted under physiological conditions or by solvolysis to a compound of formula I or formula X.

**[0038]** A "pharmaceutically acceptable active metabolite" is intended to mean a pharmacologically active product produced through metabolism in the body of a compound of formula I or formula X.

**[0039]** A "pharmaceutically acceptable solvate" is intended to mean a solvate that retains the biological effectiveness and properties of the biologically active components of compounds of formulas I and X. Examples of pharmaceutically acceptable solvates include, but are not limited to, compounds of formula I or X in combination with water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine.

**[0040]** A "pharmaceutically acceptable salt" is intended to mean a salt that retains the biological effectiveness and properties of the free acids and bases of compounds of formulas I and X and that is not biologically or otherwise undesirable. Examples of pharmaceutically acceptable salts include, but are not limited to, sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, sulfonates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates,  $\gamma$ -hydroxybutyrates, glycollates, tartrates, methane-sulfonates, propanesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates.

**[0041]** If the inventive compound is a base, the desired salt may be prepared by any suitable method known to the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid; hydrobromic acid; sulfuric acid; nitric acid; phosphoric acid; and the like, or with an organic acid, such as acetic acid; maleic acid; succinic acid; mandelic acid; fumaric acid; malonic acid; pyruvic acid; oxalic acid; glycolic acid; salicylic acid; pyranosidyl acids such as glucuronic acid

and galacturonic acid; alpha-hydroxy acids such as citric acid and tartaric acid; amino acids such as aspartic acid and glutamic acid; aromatic acids such as benzoic acid and cinnamic acid; sulfonic acids such as p-toluenesulfonic acid or ethanesulfonic acid; or the like.

**[0042]** As generally understood by those skilled in the art, an optically pure compound is one that is enantiomerically pure. As used herein, the term "optically pure" is intended to mean a compound which comprises at least a sufficient amount of a single enantiomer to yield a compound having the desired pharmacological activity. Preferably, "optically pure" is intended to mean a compound that comprises at least 90% of a single isomer (80% enantiomeric excess), preferably at least 95% (90% e.e.), more preferably at least 97.5% (95% e.e.), and most preferably at least 99% (98% e.e.).

**[0043]** Preferably in the above formulas I and X,  $R_1$  and  $R_{61}$  are H or F. In the compounds of formula I, preferably M is O.

**[0044]** Preferably  $R_{20}$  in formula I is H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group,  $-OR_{17}$ ,  $-SR_{17}$ ,  $-NR_{17}R_{18}$ ,  $-NR_{19}NR_{17}R_{18}$ , or  $-NR_{17}OR_{18}$ , wherein  $R_{17}$ ,  $R_{18}$ , and  $R_{19}$  independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group. More preferably  $R_{20}$  is the alkyl group -  $C(R_{41})(R_{42})NR_{43}R_{44}$ , wherein  $R_{41}$  and  $R_{42}$  independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group; and  $R_{43}$  and  $R_{44}$  independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group,  $-NR_{45}R_{46}$ ,  $-C(O)R_{45}$ ,  $-C(S)R_{45}$ ,  $-C(O)NR_{45}R_{46}$ ,  $-C(S)NR_{45}R_{46}$ ,  $-C(O)NR_{45}OR_{46}$ ,  $-C(S)NR_{45}OR_{46}$ ,  $-C(O)SR_{45}$ ,  $-C(O)OR_{45}$ ,  $-C(S)OR_{45}$ ,  $-C(S)SR_{45}$ ,  $-OR_{45}$ ,  $-SR_{45}$ ,  $-C(O)NR_{45}NR_{46}R_{47}$ ,  $-C(S)NR_{45}NR_{46}R_{47}$ ,  $-SOR_{45}$ ,  $-SO_2R_{45}$ ,  $-S(O)NR_{45}R_{46}$ ,  $-S(O)NR_{45}(OR_{46})$ ,  $-SO_2NR_{45}R_{46}$ ,  $-SO_2NR_{45}(OR_{46})$ , or  $-SO_3R_{45}$ , wherein  $R_{45}$ ,  $R_{46}$ , and  $R_{47}$  independently are H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or wherein any combination of  $R_{41}$ ,  $R_{42}$ ,  $R_{43}$ , and  $R_{44}$ , together with the atoms to which they are attached, form a cycloalkyl group or

a heterocycloalkyl group. Preferably at least one of  $R_{43}$  and  $R_{44}$  is  $-C(O)SR_{45}$  or  $-C(O)OR_{45}$ . Preferably  $R_{45}$  is an alkyl group, a cycloalkyl group, an aryl group, a heterocycloalkyl group, or a heteroaryl group, and more preferably a  $C_1$ - $C_{10}$  alkyl group.

**[0045]** In the compounds of formula I, preferably  $D_1$  and  $D_2$  are  $-OR_{25}$ ,  $=O$ ,  $=S$ ,  $\equiv N$ ,  $=NR_{25}$ , or  $-NR_{25}R_{26}$ , wherein  $R_{25}$  and  $R_{26}$  are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, or a heteroaryl group, or, together with the nitrogen atom to which they are bonded, form a heterocycloalkyl group, and more preferably  $D_1$  and  $D_2$  are  $=O$ . Preferably, in the compounds of formula I,  $A_1$  and  $A_2$  are C, CH, S, or S(O), and more preferably  $A_1$  and  $A_2$  are C.

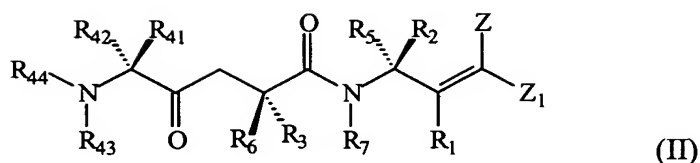
**[0046]** Preferably, in the compounds of formula I,  $B_1$  and  $B_2$  are  $NR_{17}R_{18}$ , wherein  $R_{17}$  and  $R_{18}$  are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, or an acyl group.

**[0047]** In the compounds of formula I and X, preferably  $Z$  and  $Z_1$  are independently H, an aryl group, or a heteroaryl group,  $-C(O)R_{21}$ ,  $-CO_2R_{21}$ ,  $-CN$ ,  $-C(O)NR_{21}R_{22}$ ,  $-C(O)NR_{21}OR_{22}$ ,  $-C(S)R_{21}$ ,  $-C(S)NR_{21}R_{22}$ ,  $-NO_2$ ,  $-SOR_{21}$ ,  $-SO_2R_{21}$ ,  $-SO_2NR_{21}R_{22}$ ,  $-SO(NR_{21})(OR_{22})$ ,  $-SONR_{21}$ ,  $-SO_3R_{21}$ ,  $-C(O)NR_{21}NR_{22}R_{23}$ , or  $-C(S)NR_{21}NR_{22}R_{23}$ , wherein  $R_{21}$ ,  $R_{22}$ , and  $R_{23}$  are independently H, an alkyl group, a cycloalkyl group, a heterocycloalkyl group, an aryl group, a heteroaryl group, an acyl group, or a thioacyl group, or wherein any two of  $R_{21}$ ,  $R_{22}$ , and  $R_{23}$ , together with the atom(s) to which they are bonded, form a heterocycloalkyl group, or  $Z$  and  $Z_1$ , both as defined above, together with the atoms to which they are attached, form a heterocycloalkyl group.

**[0048]** In the compounds of formula X, preferably  $R_{66}$  is  $-C(O)OR_{68}$  or  $-C(O)SR_{68}$ , wherein  $R_{68}$  is an alkyl group, a cycloalkyl group, an aryl group, a heterocycloalkyl group, or a heteroaryl group.

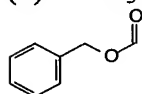
**[0049]** Particularly preferred embodiments of the present invention include the following compounds (**1-8**, **10**, and **11**) of formula II:





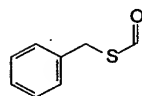
wherein  $R_1$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_{42}$ ,  $R_{43}$ , and  $Z$  are H,  $R_2$  is  $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ , and

(1)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is

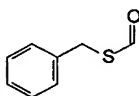


(2)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is  $\text{CH}_3\text{CH}_2\text{S}-\text{C}(=\text{O})$

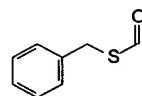
(3)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is , and  $R_{44}$  is

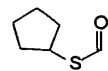


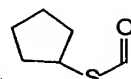
(4)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is  $\text{CH}_3\text{CH}_2\text{S}-\text{C}(=\text{O})$

(5)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is 

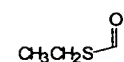
(6)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is



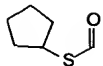
(7)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is 

(8)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is 

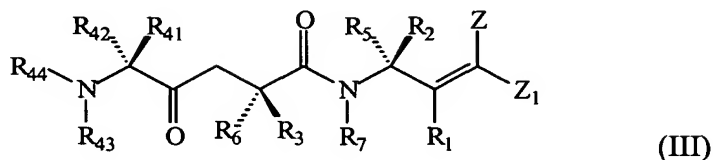
(10)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is



(11)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is

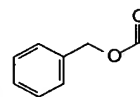


and a compound (9) of formula III:



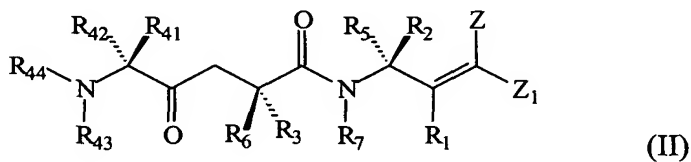
wherein  $R_1$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_{42}$ ,  $R_{43}$ , and  $Z$  are H,  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_2$  is  $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ ,

$R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is



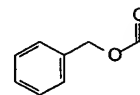
and pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates thereof.

**[0050]** The present invention is still further directed to compositions comprising at least one compound of formula II:



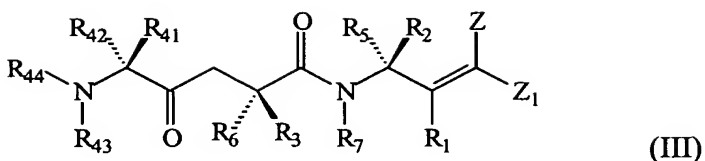
wherein  $R_1$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_{42}$ ,  $R_{43}$ , and  $Z$  are H,  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_2$  is  $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ ,

$R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ , and  $R_{44}$  is



or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof,

and at least one compound of formula III:

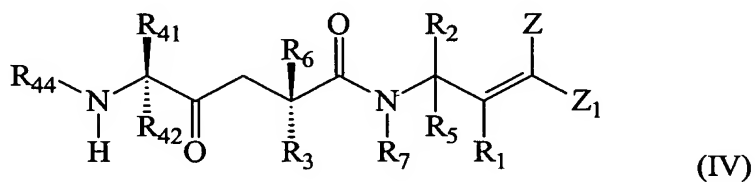


wherein  $R_1$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_{42}$ ,  $R_{43}$ , and  $Z$  are H,  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $R_2$  is  $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ ,



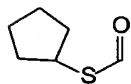
or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof.

**[0051]** Additional preferred compounds according to the present invention include the following compounds (**12** through **25**) of formula IV:

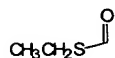


wherein  $R_1$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , and  $R_{42}$  are H,  $R_2$  is  $\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ , and

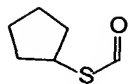
(12)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}_2\text{Ph}$ , and  $R_{44}$  is



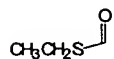
(13)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is



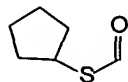
(14)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is

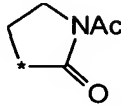


(15)  $R_3$  is  $\text{CH}_2(p\text{-CF}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is



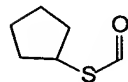
(16)  $R_3$  is  $\text{CH}_2(p\text{-CF}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is



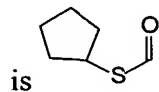
(17)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $Z$  and  $Z_1$  together form  (where \* indicates the point of attachment and the carbonyl group is cis to the  $R_1$  group),  $R_{41}$  is

$\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is 

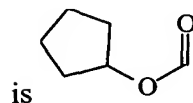
(18)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}_2\text{Ph}$ , and  $R_{44}$  is



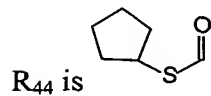
(19)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$



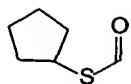
(20)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$



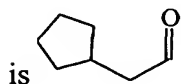
(21)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}_2\text{CH}(\text{CH}_3)_2$ , and



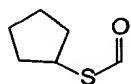
(22)  $R_3$  is  $\text{CH}_2\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{C}(\text{CH}_3)_3$ , and  $R_{44}$  is



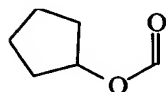
(23)  $R_3$  is  $\text{CH}_2(p\text{-CH}_3)\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is



(24)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is cyclohexyl, and  $R_{44}$  is



(25)  $R_3$  is  $\text{CH}_2(p\text{-F})\text{Ph}$ ,  $Z$  is H,  $Z_1$  is  $\text{CO}_2\text{CH}_2\text{CH}_3$ ,  $R_{41}$  is  $\text{CH}(\text{CH}_3)_2$ , and  $R_{44}$  is



and pharmaceutically acceptable prodrugs, salts, active metabolites, or solvates thereof.

[0052] The present invention is even further directed to methods of inhibiting picornaviral 3C protease activity, comprising contacting the protease with an effective amount of a compound of formula I or X or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof. For example, one can inhibit picornaviral 3C protease activity in mammalian tissue by administering a compound of formula I or X or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof. More particularly, the present invention is directed to methods of inhibiting rhinoviral protease activity.

[0053] The activity of the inventive compounds as inhibitors of picornaviral 3C protease activity may be measured by any of the methods available to those skilled in the art, including in vivo and in vitro assays. An example of a suitable assay for activity measurements is the Antiviral HI-HeLa Cell Culture Assay, described herein.

**[0054]** Administration of the compounds of the formula I or X, or their pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates, may be performed according to any of the accepted modes of administration available to those skilled in the art. Illustrative examples of suitable modes of administration include, but are not limited to, oral, nasal, parenteral, topical, transdermal, and rectal.

**[0055]** The inventive compounds of formulas I and X, and their pharmaceutically acceptable prodrugs, salts, active metabolites, and solvates, may be administered as a pharmaceutical composition in any suitable pharmaceutical form recognizable to the skilled artisan. Suitable pharmaceutical forms include, but are not limited to, solid, semisolid, liquid, or lyophilized formulations, such as tablets, powders, capsules, suppositories, suspensions, and aerosols. The pharmaceutical composition may also include suitable excipients, diluents, vehicles, and carriers, as well as other pharmaceutically active agents, depending upon the intended use.

**[0056]** Acceptable methods of preparing suitable pharmaceutical forms of the pharmaceutical compositions are known to those skilled in the art. For example, pharmaceutical preparations may be prepared following conventional techniques of the pharmaceutical chemist involving steps such as mixing, granulating, and compressing when necessary for tablet forms, or mixing, filling, and dissolving the ingredients as appropriate, to give the desired products for oral, parenteral, topical, intravaginal, intranasal, intrabronchial, intraocular, intraaural, and/or rectal administration.

**[0057]** Solid or liquid pharmaceutically acceptable carriers, diluents, vehicles, or excipients may be employed in the pharmaceutical compositions. Illustrative solid carriers include starch, lactose, calcium sulphate dihydrate, terra alba, sucrose, talc, gelatin, pectin, acacia, magnesium stearate, and stearic acid. Illustrative liquid carriers may include syrup, peanut oil, olive oil, saline solution, and water. The carrier or diluent may include a suitable prolonged-release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft

gelatin capsule, sterile injectable liquid (e.g. solution), or a nonaqueous or aqueous liquid suspension.

**[0058]** A dose of the pharmaceutical composition contains at least a therapeutically effective amount of the active compound (i.e., a compound of formula I or X or a pharmaceutically acceptable prodrug, salt, active metabolite, or solvate thereof) and preferably is made up of one or more pharmaceutical dosage units. The selected dose may be administered to a mammal, for example, a human patient, in need of treatment mediated by inhibition of 3C protease activity, by any known method of administering the dose including topical, for example, as an ointment or cream; orally; rectally, for example, as a suppository; parenterally by injection; or continuously by intravaginal, intranasal, intrabronchial, intraaural, or intraocular infusion.

**[0059]** A "therapeutically effective amount" is intended to mean that amount of a compound of formula I or X that, when administered to a mammal in need thereof, is sufficient to effect treatment for disease conditions alleviated by the inhibition of the activity of one or more picornaviral 3C proteases, such as human rhinoviruses, human poliovirus, human coxsackieviruses, encephalomyocarditis viruses, menigovirus, and hepatitis A virus. The amount of a given compound of formula I or X that will correspond to a "therapeutically effective amount" will vary depending upon factors such as the particular compound, the disease condition and the severity thereof, the identity of the mammal in need thereof, but it can nevertheless be readily determined by one of skill in the art.

**[0060]** "Treating" or "treatment" is intended to mean at least the mitigation of a disease condition in a mammal, such as a human, that is alleviated by the inhibition of the activity of one or more picornaviral 3C proteases, such as human rhinoviruses, human poliovirus, human coxsackieviruses, encephalomyocarditis viruses, menigovirus, and hepatitis A virus, and includes:

(a) prophylactic treatment in a mammal, particularly when the mammal is found to be predisposed to having the disease condition but not yet diagnosed as

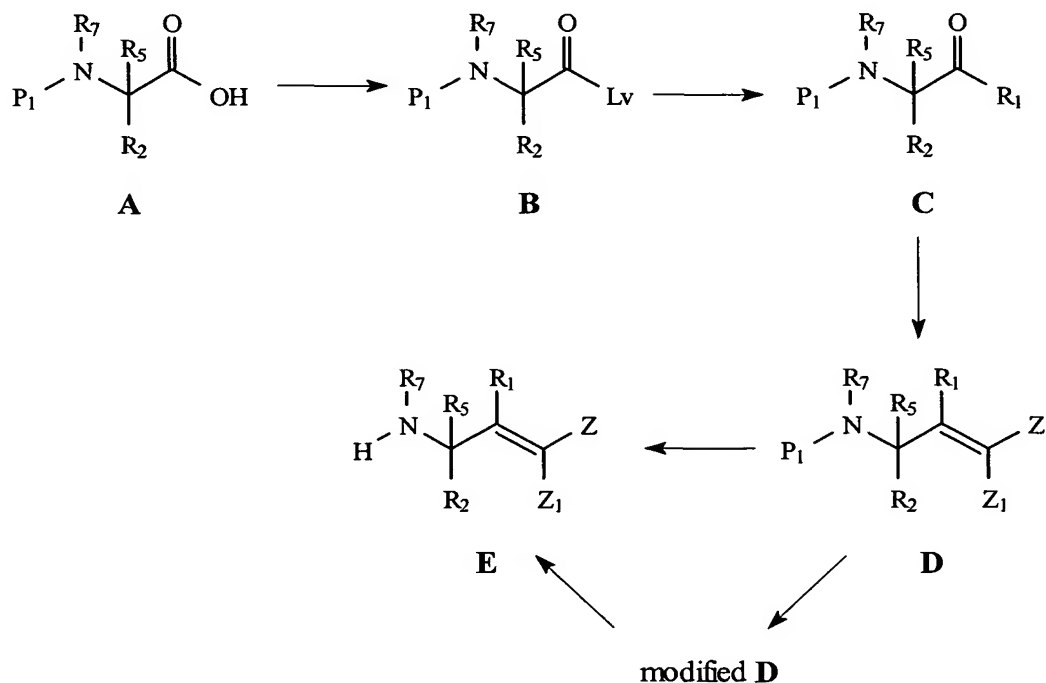
having it;

- (b) inhibiting the disease condition; and/or
- (c) alleviating, in whole or in part, the disease condition.

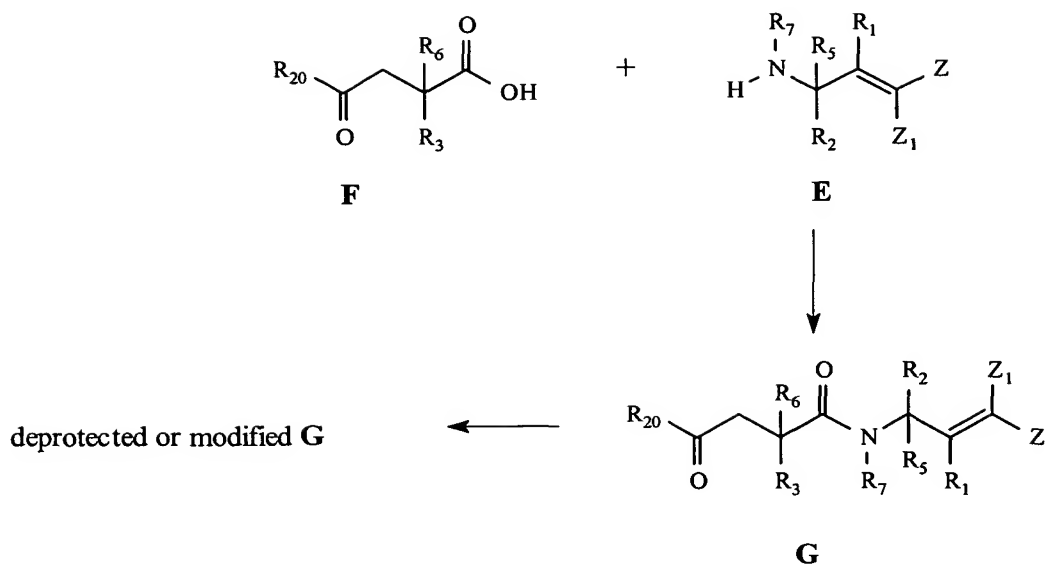
**[0061]** The inventive compounds, and their salts, solvates, active metabolites, and prodrugs, may be prepared by employing the techniques available in the art using starting materials that are readily available. Certain novel and exemplary methods of preparing the inventive compounds are described below.

**[0062]** Preferably, the inventive compounds of formula I are prepared by the methods of the present invention, including the general methods shown below. In each of these general methods, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>20</sub>, R<sub>41</sub>, R<sub>42</sub>, Z, and Z<sub>1</sub> are as defined above (for formulae I, II, III, IV, and X).

**General Method I**



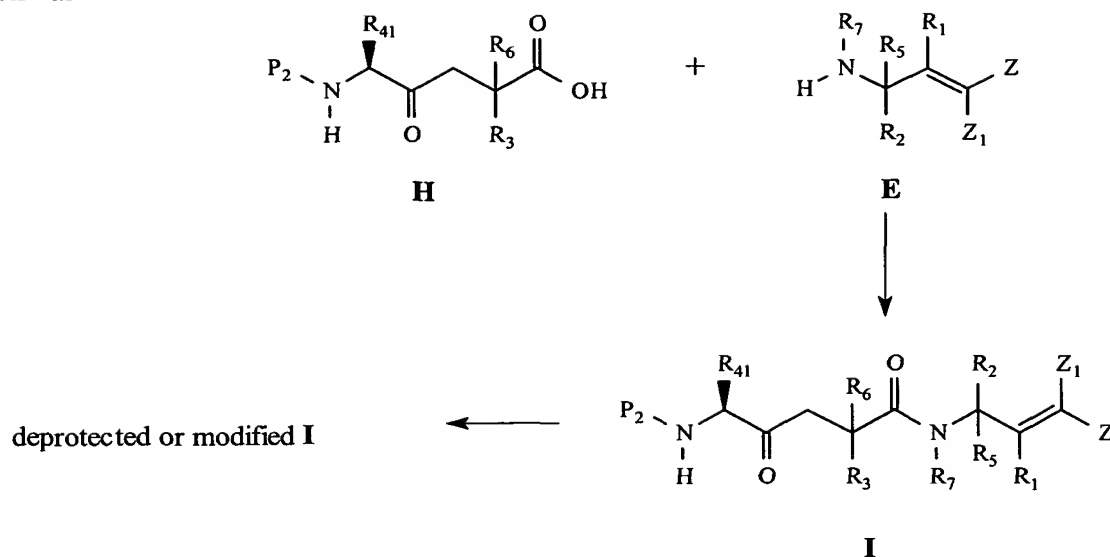




**[0063]** In General Method I, amino acid A, where P1 is an appropriate protecting group for nitrogen, is converted to carbonyl derivative B, where "Lv" is a leaving group. Compound B is subjected to a reaction where "Lv" is replaced by R1 to give derivative C. Derivative C is then transformed into unsaturated product D. Unsaturated compound D is deprotected to give free amine (or salt thereof) E, or modified one or more times at R2, R5, Z, and/or Z1 to give one or more modified D compounds. Modified D is then deprotected to give amine (or salt thereof) E.

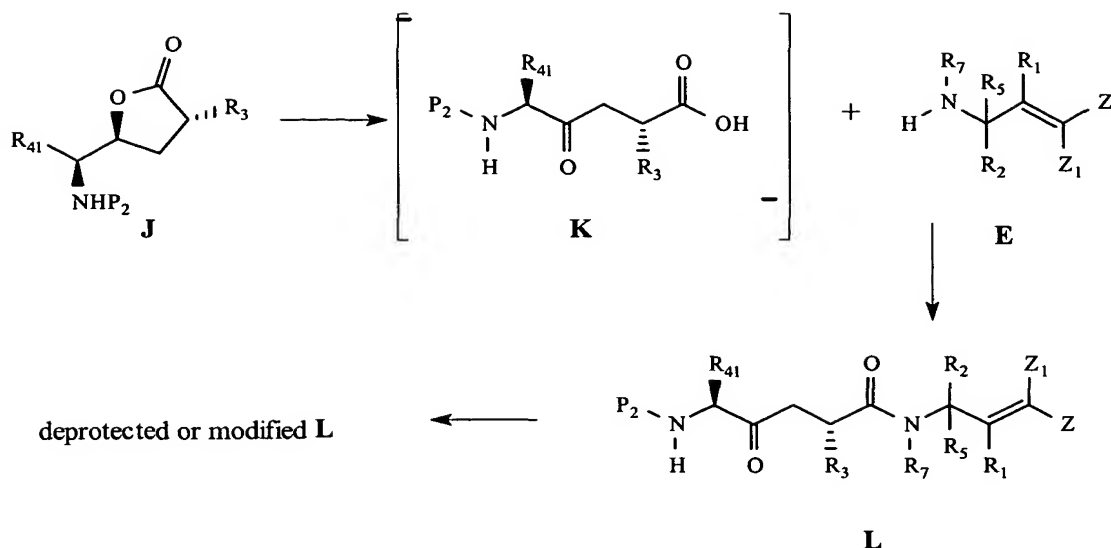
**[0064]** Amine E is subsequently subjected to an amide-forming reaction with carboxylic acid F (for which the preparation of representative examples is described below) to give final product G. If protecting groups were used on any R groups ( $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{R}_3$ ,  $\text{R}_5$ ,  $\text{R}_6$ ,  $\text{R}_7$ , and/or  $\text{R}_{20}$ ), on Z and/or on  $\text{Z}_1$ , product G is deprotected and/or further modified to yield "deprotected or modified G."

General Method II



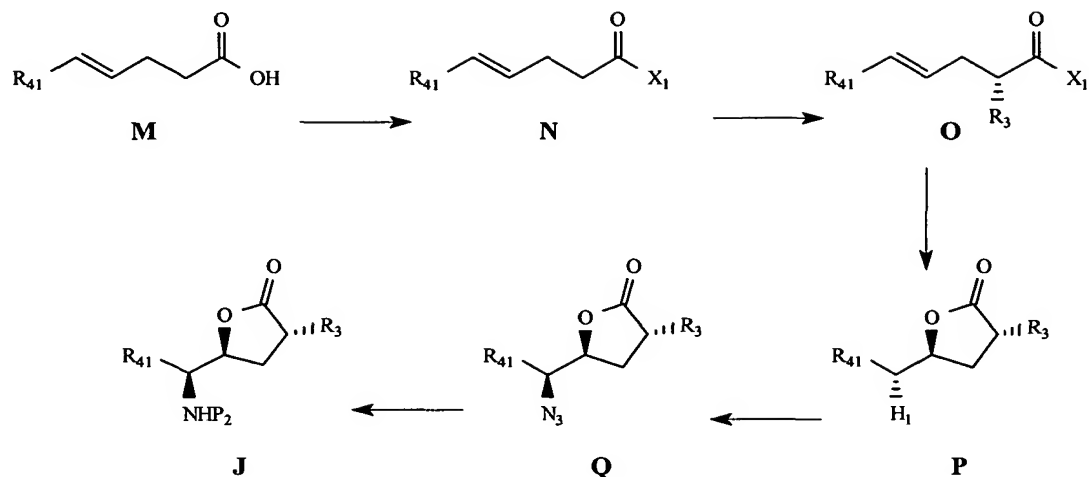
[0065] In General Method II, amine **E**, which can be prepared as described in General Method I, is subjected to an amide-forming reaction with carboxylic acid **H**, where  $P_2$  is an appropriate protecting group for nitrogen, and where at least one of  $R_3$  and  $R_6$  is H, to give final product **I**. Carboxylic acid **H** can be prepared as a mixture of diastereomers as described in Harbeson, S.L., Rich, D.H., *J. Med. Chem.* **1989**, 32, 1378, the disclosure of which is incorporated herein by reference. The  $P_2$  protecting group, along with any additional protecting groups that were used on any R groups ( $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_6$ ,  $R_7$ , and/or  $R_{41}$ ), on Z and/or on  $Z_1$ , is subsequently deprotected and/or further modified to yield "deprotected or modified **I**."

General Method III



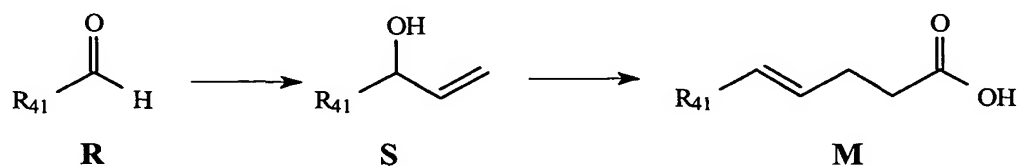
[0066] In General Method III, optically active lactone **J**, where  $P_2$  is an appropriate protecting group for nitrogen, is transformed by a two-step procedure (basic hydrolysis and subsequent oxidation) into carboxylic acid **K**. Lactone **J** can be prepared by the method described in General Method IV below and by many literature methods including, but not limited to, those described in the following: (a) Herold, P.; Duthaler, R.; Rihs, G.; Angst, C., *J. Org. Chem.* **1989**, *54*, 1178; (b) Bradbury, R. H.; Revill, J. M.; Rivett, J. E.; Waterson, D., *Tetrahedron Lett.* **1989**, *30*, 3845; (c) Bradbury, R. H.; Major, J. S.; Oldham, A. A.; Rivett, J. E.; Roberts, D. A.; Slater, A. M.; Timms, D.; Waterson, D., *J. Med. Chem.* **1990**, *33*, 2335; (d) Wuts, P. G.; Ritter, A. R.; Pruitt, L. E., *J. Org. Chem.* **1992**, *57*, 6696; (e) Jones, D. M.; Nilsson, B.; Szelke, M., *J. Org. Chem.* **1993**, *58*, 2286; (f) Pégrier, L.; Larchevéque, M., *Tetrahedron Lett.* **1995**, *36*, 2753; (g) Dondoni, A.; Perrone, D.; Semola, M. T., *J. Org. Chem.* **1995**, *60*, 7927, all of which are incorporated herein by reference. Carboxylic acid **K** is not isolated in pure form, but is subjected to an amide-forming reaction with amine **E**, which can be prepared as described in General Method I, to provide final product **L**. The  $P_2$  protecting group, along with any additional protecting groups that were used on any R groups ( $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_5$ ,  $R_7$ , and/or  $R_{41}$ ), on Z and/or on  $Z_1$ , is subsequently deprotected and/or further modified to yield "deprotected or modified **L**."

General Method IV



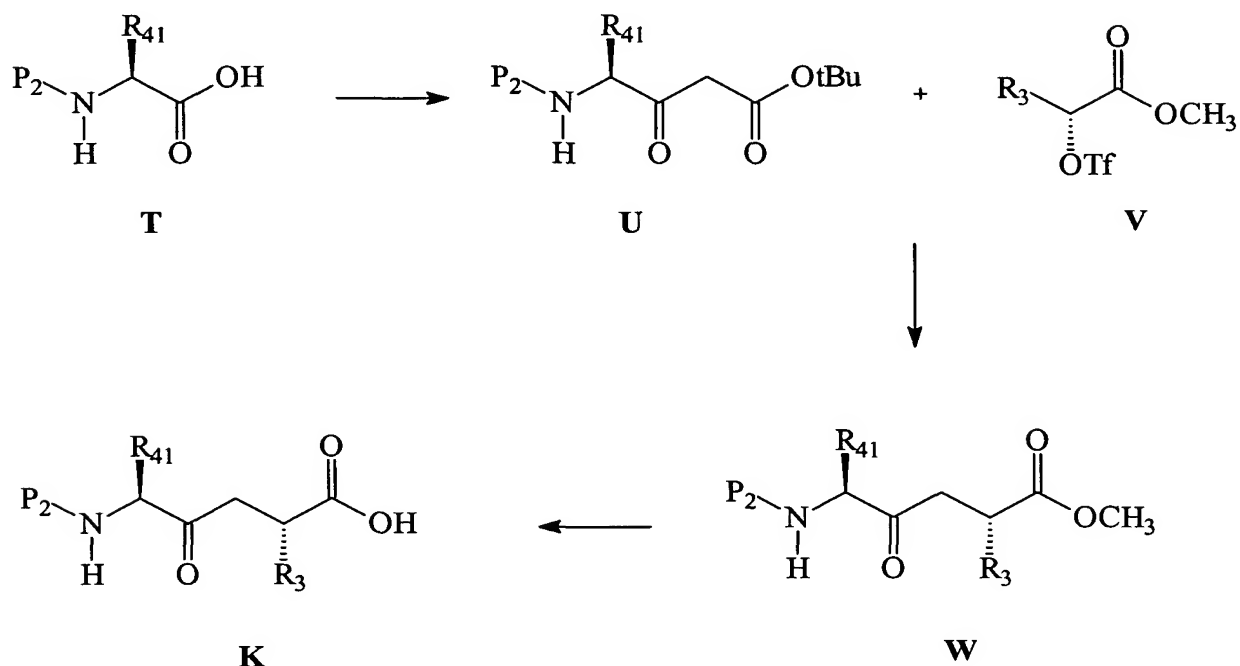
[0067] Lactone **J** may be prepared in optically active form by General Method IV (see: (a) Herold, P.; Duthaler, R.; Rihs, G.; Angst, C., *J. Org. Chem.* **1989**, *54*, 1178; (b) Bradbury, R. H.; Revill, J. M.; Rivett, J. E.; Waterson, D., *Tetrahedron Lett.* **1989**, *30*, 3845; (c) Bradbury, R. H.; Major, J. S.; Oldham, A. A.; Rivett, J. E.; Roberts, D. A.; Slater, A. M.; Timms, D.; Waterson, D., *J. Med. Chem.* **1990**, *33*, 2335). A  $\gamma,\delta$ -unsaturated carboxylic acid **M**, which incorporates  $R_{41}$ , is transformed into the corresponding acid chloride (not shown). This acid chloride is subjected to an amide-forming reaction with a chiral amine or a chiral oxazolidone to provide derivative **N** (in which  $X_1$  is a chiral amine or a chiral oxazolidone). Compound **N** is subsequently deprotonated, and the resulting enolate is diastereoselectively alkylated with an electrophile corresponding to  $R_3$  to provide compound **O**. This material is then subjected to a halolactonization reaction to provide halo-lactone **P**, in which  $H_1$  is Br or I. Halo-lactone **P** is subsequently transformed into azide **Q**, and this material is then converted into lactone **J**, where  $P_2$  is an appropriate protecting group for nitrogen.

General Method V



[0068]  $\gamma,\delta$ -Unsaturated carboxylic acid **M** may be prepared by General Method V (see: Herold, P.; Duthaler, R.; Rihs, G.; Angst, C., *J. Org. Chem.* **1989**, *54*, 1178). An aldehyde **R**, which incorporates  $R_{41}$ , is coupled with vinylmagnesium bromide to give alcohol **S**. Alcohol **S** is then transformed into  $\gamma,\delta$ -unsaturated carboxylic acid **M** by a three step procedure as follows: (i) treatment with diethyl malonate and catalytic  $Ti(OEt)_4$  at 160 °C for 1 hour, (ii) heating at 190 °C for 4 hours, and (iii) hydrolysis with ethanolic KOH at reflux.

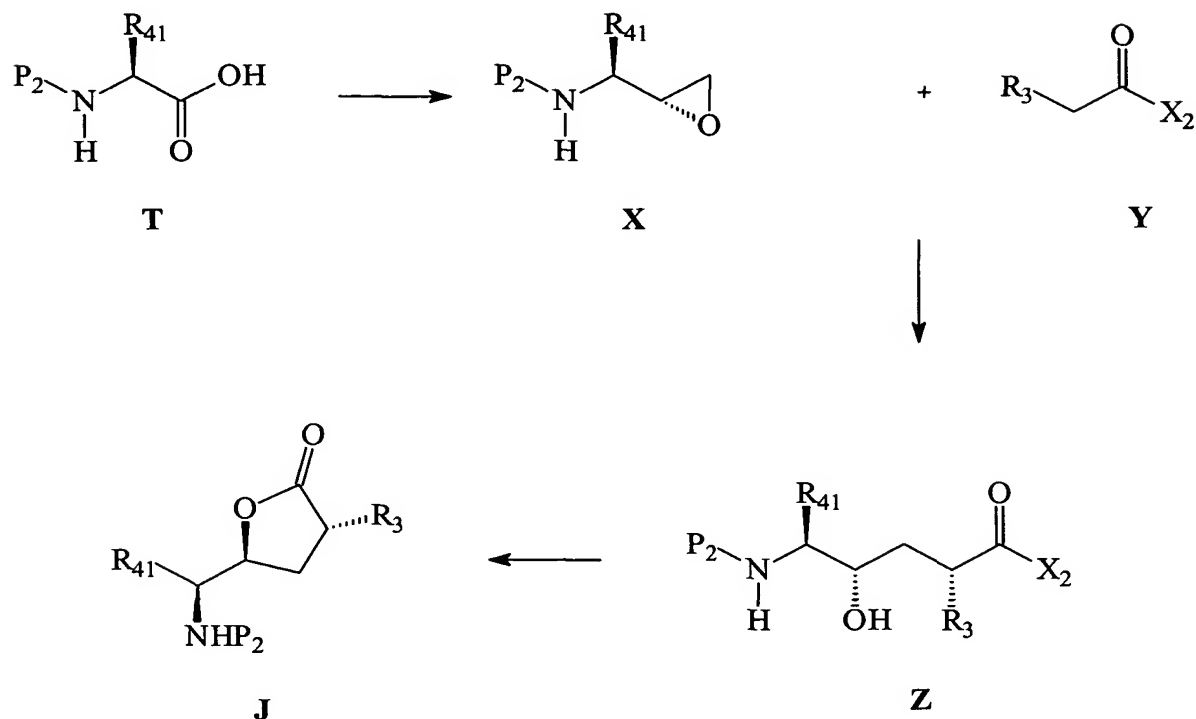
General Method VI



[0069] Carboxylic acid **K** also may be prepared by General Method VI (see: Hoffman, R. V., Tao, J., *Tetrahedron*, **1997**, *53*, 7119, which document is entirely incorporated herein by reference). An amino acid **T**, which incorporates  $R_{41}$  and where  $P_2$  is an appropriate protecting group for nitrogen, is transformed into  $\beta$ -ketoester **U**. Compound **U** is deprotonated, and the resulting anion is condensed with triflate **V**, which incorporates  $R_3$ . The coupling product thus obtained is

treated with trifluoroacetic acid to provide ketoester **W**, and this material is subsequently hydrolyzed to afford carboxylic acid **K**. Triflate **V**, in turn, may be prepared from the corresponding alcohol by treatment with trifluoromethanesulfonic anhydride and 2,6-lutidine.

#### General Method VII



[0070] Lactone **J** also may be prepared by General Method VII (see: (a) Askin, D., Wallace, M. A., Vacca, J. P., Reamer, R. A., Volante, R. P., Shinkai, I. *J. Org. Chem.* **1992**, 57, 2771 (b) McWilliams, J. C., Armstrong, J. D., Zheng, N., Bhupathy, M., Volante, R. P., Reider, P. J., *J. Am. Chem. Soc.* **1996**, 118, 11970; each of these documents is entirely incorporated herein by reference). An amino acid **T**, which incorporates  $R_{41}$  and where  $P_2$  is an appropriate protecting group for nitrogen, is transformed into epoxide **X** (single diastereomer) by the method described in: Luly, J. R., Dellaria, J. F., Plattner, J. J., Soderquist, J. L., Yi, N., *J. Org. Chem.* **1987**, 52, 1487, the disclosure of which is entirely incorporated herein by reference. Alternatively, **X** may be prepared from **T** as a mixture of

diastereomers as described in the "Examples" section of this document. Epoxide **X** is condensed with the anion derived from compound **Y**, which incorporates  $R_3$  and in which  $X_2$  is a chiral auxiliary [including (1*S*,2*R*)-1-aminoindan-2-ol acetonide] to afford coupling product **Z**. If **X** was utilized as a mixture of diastereomers, the diastereomer of **Z** depicted below is purified from other **Z** isomers (if any) produced in the coupling reaction. This material is subsequently cyclized under acidic conditions to provide lactone **J**. Compound **Y** may be prepared from the corresponding carboxylic acid (not shown) by the method outlined in: Askin, D., Wallace, M. A., Vacca, J. P., Reamer, R. A., Volante, R. P., Shinkai, I., *J. Org. Chem.* **1992**, *57*, 2771.

[0071] Suitable protecting groups for nitrogen are recognizable to those skilled in the art and include, but are not limited to benzyloxycarbonyl, t-butoxycarbonyl, 9-fluorenylmethoxycarbonyl, p-methoxybenzyloxycarbonyl, trifluoroacetamide, and p-toluenesulfonyl. Suitable protecting groups for oxygen are recognizable to those skilled in the art and include, but are not limited to -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>3</sub>, tBu, -CH<sub>2</sub>Ph, -CH<sub>2</sub>CH=CH<sub>2</sub>, -CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>Si(CH<sub>3</sub>)<sub>3</sub>, and -CH<sub>2</sub>CCl<sub>3</sub>. Other examples of suitable protecting groups for nitrogen or oxygen can be found in T. Green & P. Wuts, Protective Groups in Organic Synthesis (2nd ed. 1991), the disclosure of which is incorporated herein by reference.

[0072] Suitable leaving groups also are recognizable to those skilled in the art and include, but are not limited to, Cl, Br, I, sulfonates, O-alkyl groups,

[0073] Other examples of suitable leaving groups are described in J. March, Advanced Organic Chemistry, Reactions, Mechanisms, and Structure (4th ed. 1992) at pages 205, 351-56, 642-43, 647, 652-53, 666, 501, 520-21, 569, 579-80, 992-94, 999-1000, 1005, and 1008, the disclosure of which is incorporated herein by reference.

## EXAMPLES

[0074] Examples of the processes used to make several of the compounds of formula I are set forth below. These Examples are intended to illustrate the present invention without limiting it. The structures of the compounds of the following examples were confirmed by one or more of the following: proton magnetic resonance spectroscopy, infrared spectroscopy, elemental microanalysis, mass spectrometry, thin layer chromatography, melting point, and boiling point.

[0075] Proton magnetic resonance ( $^1\text{H}$  NMR) spectra were determined using a Varian UNITYplus 300 spectrometer operating at a field strength of 300 megahertz (MHZ). Chemical shifts are reported in parts per million (ppm,  $\delta$ ) downfield from an internal tetramethylsilane standard. Alternatively,  $^1\text{H}$  NMR spectra were referenced to residual protic solvent signals as follows:  $\text{CHCl}_3 = 7.26$  ppm; DMSO = 2.49 ppm,  $\text{C}_6\text{HD}_5 = 7.15$  ppm. Peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; br, broad resonance; m, multiplet. Coupling constants (J) are given in hertz (Hz). Infrared absorption (IR) spectra were obtained using a Perkin-Elmer 1600 series FTIR spectrometer. Elemental microanalyses were performed by Atlantic Microlab Inc., Norcross, GA and gave results for the elements stated within  $\pm 0.4\%$  of the theoretical values.

[0076] Flash column chromatography was performed using Silica gel 60 (Merck Art 9385). Analytical thin layer chromatography (TLC) was performed using precoated sheets of Silica 60 F254 (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected. All reactions were performed in septum-sealed flasks under a slight positive pressure of argon unless otherwise noted. All commercial reagents were used as received from their respective suppliers with the following exceptions. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl prior to use. Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) was distilled from calcium hydride prior to use. Anhydrous lithium chloride was prepared by heating at  $110^\circ\text{C}$  under vacuum overnight. Et<sub>2</sub>O refers to diethyl ether. DMF refers to N,N-dimethylformamide. DMSO refers to dimethylsulfoxide. Other abbreviations include: CH<sub>3</sub>OH (methanol), EtOH



(ethanol), EtOAc (ethyl acetate), DME (ethylene glycol dimethyl ether), Ac (acetyl), Me (methyl), Et (ethyl), Ph (phenyl), Bn (benzyl), CyPentyl (cyclopentyl), Tr (triphenylmethyl), CBZ (benzyloxycarbonyl), BOC (tert-butoxycarbonyl), Gln (glutamine), Leu (leucine), Phe (phenylalanine), Val (valine). Additionally, "L" represents naturally occurring amino acids, "D" represents unnatural amino acids, and "DL" represents a racemic mixture of the two.

**[0077]** A simplified naming system employing amino acid abbreviations is used to identify some intermediates and final products. When naming compounds, italicized amino acid abbreviations represent modifications at the C-terminus of that residue where the following apply:

1. acrylic acid esters are reported as "E" (trans) propenoates;
2. acrylamides are reported as "E" (trans) propenamides; and
3. N-acetyl-pyrrolidin-2-ones are reported as "E" (trans) 1-acetyl-3-methylene-pyrrolidin-2-ones.

**[0078]** In addition, the terminology "AA<sub>1</sub>Ψ[COCH<sub>2</sub>]-AA<sub>2</sub>" indicates that, for any peptide sequence, two amino acids (AA<sub>1</sub> and AA<sub>2</sub>) linked by an amide bond are replaced by a ketomethylene dipeptide isostere moiety.

**Example 1 - Preparation of a Mixture of Compound 1 and Compound 9 (~1:1): Ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate [BOC-L-(Tr-Gln)]-N(OMe)Me.**

**[0079]** Isobutyl chloroformate (4.77 mL, 36.8 mmol, 1.0 equiv) was added to a solution of [BOC-L-(Tr-Gln)]-OH (18.7 g, 36.7 mmol, 1 equiv) and 4-methylmorpholine (8.08 mL, 73.5 mmol, 2.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (250 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 20 minutes, then *N,O*-dimethylhydroxylamine hydrochloride (3.60 g, 36.7 mmol, 1.0 equiv) was added. The resulting solution was stirred at 0 °C for 20 minutes and at 23 °C for 2 hours, and then was partitioned between water (150 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, and were concentrated.

Purification of the residue by flash column chromatography (gradient elution, 40% → 20% hexanes in EtOAc) provided [BOC-L-(Tr-Gln)]-N(OMe)Me (16.1 g, 82%) as a white foam:  $R_f$  = 0.22 (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3411, 3329, 3062, 1701, 1659;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.42 (s, 9H), 1.63-1.77 (m, 1H), 2.06-2.17 (m, 1H), 2.29-2.43 (m, 2H), 3.17 (s, 3H), 3.64 (s, 3H), 4.73 (s, br, 1H), 5.38-5.41 (m, 1H), 7.20-7.31 (m, 15H); Anal. ( $\text{C}_{31}\text{H}_{37}\text{N}_3\text{O}_5$ ) C, H, N.

#### **Preparation of Intermediate [BOC-L-(Tr-Gln)]-H.**

[0080] Diisobutylaluminum hydride (50.5 mL of a 1.5 M solution in toluene, 75.8 mmol, 2.5 equiv) was added to a solution of [BOC-L-(Tr-Gln)]-N(OMe)Me (16.1 g, 30.3 mmol, 1 equiv) in THF at  $-78^\circ\text{C}$ , and the reaction mixture was stirred at  $-78^\circ\text{C}$  for 4 hours. Methanol (4 mL) and 1.0 M HCl (10 mL) were added sequentially, and the mixture was warmed to  $23^\circ\text{C}$ . The resulting suspension was diluted with  $\text{Et}_2\text{O}$  (150 mL) and was washed with 1.0 M HCl (3 x 100 mL), half-saturated  $\text{NaHCO}_3$  (100 mL), and water (100 mL). The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated to give crude [BOC-L-(Tr-Gln)]-H (13.8 g, 97%) as a white solid: mp =  $114\text{--}116^\circ\text{C}$ ;  $R_f$  = 0.42 (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3313, 1697, 1494;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.44 (s, 9H), 1.65-1.75 (m, 1H), 2.17-2.23 (m, 1H), 2.31-2.54 (m, 2H), 4.11 (s, br, 1H), 5.38-5.40 (m, 1H), 7.11 (s, 1H), 7.16-7.36 (m, 15H), 9.45 (s, 1H).

#### **Preparation of Intermediate Ethyl-3-[BOC-L-(Tr-Gln)]-E-Propenoate.**

[0081] Sodium bis(trimethylsilyl)amide (22.9 mL of a 1.0 M solution in THF, 22.9 mmol, 1.0 equiv) was added to a solution of triethyl phosphonoacetate (5.59 g, 22.9 mmol, 1.0 equiv) in THF (200 mL) at  $-78^\circ\text{C}$ , and the resulting solution was stirred for 20 minutes at that temperature. Crude [BOC-L-(Tr-Gln)]-H (10.8 g, 22.9 mmol, 1 equiv) in THF (50 mL) was added via cannula, and the reaction mixture was stirred for 2 hours at  $-78^\circ\text{C}$ , warmed to  $0^\circ\text{C}$  for 10 minutes, and partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated. Purification of the residue by flash column chromatography (40%

EtOAc in hexanes) provided ethyl-3-[BOC-L-(Tr-*Gln*)]-E-propenoate (10.9 g, 88%) as a white foam:  $R_f$  = 0.60 (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3321, 1710;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.27 (t, 3H,  $J$  = 7.2), 1.42 (s, 9H), 1.70-1.78 (m, 1H), 1.80-1.96 (m, 1H), 2.35 (t, 2H,  $J$  = 7.0), 4.18 (q, 2H,  $J$  = 7.2), 4.29 (s, br, 1H), 4.82-4.84 (m, 1H), 5.88 (dd, 1H,  $J$  = 15.7, 1.6), 6.79 (dd, 1H,  $J$  = 15.7, 5.3), 6.92 (s, 1H), 7.19-7.34 (m, 15H); Anal. ( $\text{C}_{33}\text{H}_{38}\text{N}_2\text{O}_5$ ) C, H, N.

**Preparation of Intermediate Ethyl-3-[CBZ-L-Leu $\Psi$ [COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0082] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-(Tr-*Gln*)]-E-propenoate (0.091 g, 0.17 mmol, 1 equiv) in 1,4-dioxane (3 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 1.5 hours, then was concentrated under reduced pressure to afford crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl as a viscous oil. This material was dissolved in  $\text{CH}_2\text{Cl}_2$  (6 mL) and CBZ-L-Leu $\Psi$ [COCH<sub>2</sub>]-D/L-Phe-OH (0.068 g, 0.17 mmol, 1.0 equiv) [prepared as described in: Harbeson, S. L., Rich, D. H., *J. Med. Chem.* **1989**, 32, 1378], 1-hydroxybenzotriazole hydrate (0.030 g, 0.22 mmol, 1.3 equiv), 4-methylmorpholine (0.055 mL, 0.50 mmol, 3.0 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.042 g, 0.22 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 21 hours at 23 °C and then partitioned between water (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  and were concentrated. Purification of the residue by flash column chromatography (50% EtOAc in hexanes) provided ethyl-3-[CBZ-L-Leu $\Psi$ [COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.040 g, 28%) as a white foam:  $R_f$  = 0.44 (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3317, 1712, 1667;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , approximately 1:1 mixture of diastereomers)  $\delta$  0.84-0.91 (m), 1.20-1.31 (m), 1.51-1.58 (m), 1.73-1.96 (m), 2.29-2.39 (m), 2.51-2.72 (m), 2.94-3.07 (m), 4.11-4.30 (m), 4.47-4.50 (m), 4.84 (d,  $J$  = 7.8 Hz), 4.94-5.08 (m), 5.09 (s), 5.30 (d,  $J$  = 7.2 Hz), 5.48 (d,  $J$  = 14.3 Hz), 5.72-5.95 (m), 6.55-7.01 (m), 7.14-7.61 (m), 8.02-8.05 (m); Anal. ( $\text{C}_{52}\text{H}_{57}\text{N}_3\text{O}_7 \cdot 0.75\text{H}_2\text{O}$ ) C, H, N.

**Preparation of Product Ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-Gln)-E-Propenoate.**

[0083] Triisopropylsilane (0.10 mL) and trifluoroacetic acid (3 mL) were added sequentially to a solution of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-Gln)]-E-propenoate (0.035 g, 0.042 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C, then carbon tetrachloride (4 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-Gln)-E-propenoate (0.014 g, 56%) as a white foam: *R*<sub>f</sub> = 0.39 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3306, 1712, 1661; <sup>1</sup>H NMR (CDCl<sub>3</sub>, approximately 1:1 mixture of diastereomers) δ 0.87-0.93 (m), 1.24-1.33 (m), 1.39-1.96 (m), 2.17-2.21 (m), 2.58-2.79 (m), 2.87-3.09 (m), 4.10-4.27 (m), 4.44 (s, br), 4.55 (s, br), 5.01-5.10 (m), 5.14-5.69 (m), 5.79 (s), 5.82-5.91 (m), 6.13 (d, *J* = 7.5 Hz), 6.42 (s, br), 6.59 (dd, *J* = 16.0, 7.5 Hz), 6.74 (dd, *J* = 15.6, 4.7 Hz), 7.16-7.39 (m); Anal. (C<sub>33</sub>H<sub>43</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Example 2 - Preparation of Compound 1: Ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate trans-7-Methyl-oct-4-enoic Acid.**

[0084] A solution of isovaleraldehyde (8.61 g, 100 mmol, 1 equiv) in THF (30 mL) was added dropwise via addition funnel to a solution of vinylmagnesium bromide (100 mL of a 1.0 M solution in THF, 100 mmol, 1.0 equiv) in THF (150 mL) at 0 °C. After the addition was completed, the reaction mixture was stirred for 30 minutes at 0 °C, and then was partitioned between saturated NH<sub>4</sub>Cl (150 mL) and Et<sub>2</sub>O (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to afford a yellow oil. This material was combined (neat) with diethyl malonate (16.7 mL, 110 mmol, 1.1 equiv) and Ti(OEt)<sub>4</sub> (2.10 mL, 10.0 mmol, 0.10 equiv), and the combination was heated to 160 °C for 1 hour (distilling out EtOH as it was formed). The reaction mixture was then maintained at 190 °C for 4 hours and then cooled to 60 °C. EtOH (50 mL) and 6.0 M KOH (50 mL)

were added sequentially, and the brown reaction mixture was refluxed for 4 hours. After cooling to 23 °C, the reaction mixture was filtered through a medium frit, and the filtrate was partitioned between water (150 mL) and Et<sub>2</sub>O (2 x 150 mL). The aqueous layer was then acidified to pH = 2 (as indicated by pH paper) with concentrated HCl and extracted with a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was distilled at reduced pressure to afford trans-7-methyl-oct-4-enoic acid (4.62 g, 30%) as a colorless liquid: bp: 115-120 °C (1 torr); <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 0.86 (d, 6H, *J* = 5.5), 1.51-1.65 (m, 1H), 1.87 (t, 2H, *J* = 6.5), 2.22-2.38 (m, 2H), 2.40-2.45 (m, 2H), 5.34-5.52 (m, 2H); Anal. (C<sub>9</sub>H<sub>16</sub>O<sub>2</sub>) C, H.

**Preparation of Intermediate trans-7-Methyl-oct-4-enoic Acid  
(2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0085] Oxalyl chloride (2.71 mL, 31.1 mmol, 1.05 equiv) was added to a solution of trans-7-methyl-oct-4-enoic acid (4.62 g, 29.6 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.012 equiv) in benzene (100 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 2 hours and then concentrated under reduced pressure. The resulting oil was dissolved in THF (20 mL) and added via cannula to a solution of (1*R*,2*R*)-(-)-pseudoephedrine (4.45 g, 26.9 mmol, 0.91 equiv) and triethylamine (4.50 mL, 32.3 mmol, 1.1 equiv) in THF (200 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 minutes and then partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and EtOAc (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (gradient elution 40 → 50% EtOAc in hexanes) to afford trans-7-methyl-oct-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (7.55 g, 93%) as a viscous oil: *R*<sub>f</sub> = 0.27 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3382, 1622; <sup>1</sup>H NGR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.87 (d, *J* = 6.5), 0.99 (d, *J* = 6.8), 1.11 (d, *J* = 7.2), 1.53-1.66 (m), 1.86 (t, *J* = 6.1), 2.26-2.54 (m), 2.82 (s), 2.92 (s), 3.99-4.04 (m), 4.29 (s, br), 4.42-4.47 (m), 4.56-4.62 (m), 5.37-5.51 (m), 7.26-7.36 (m); Anal. (C<sub>19</sub>H<sub>29</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate trans-2*S*-Benzyl-7-methyl-oct-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0086] *n*-Butyllithium (52.6 mL of a 1.6 M solution in hexanes, 84.2 mmol, 2.1 equiv) was added to a suspension of anhydrous lithium chloride (11.9 g, 282 mmol, 7.0 equiv) and diisopropylamine (12.7 mL, 90.3 mmol, 2.25 equiv) in THF (300 mL) at -78 °C. The reaction mixture was stirred for 20 minutes at -78 °C and then maintained at 0 °C for 5 minutes and subsequently cooled again to -78 °C. trans-7-Methyl-oct-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (12.2 g, 40.1 mmol, 1 equiv) in THF (40 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1 hour, maintained at 0 °C for 15 minutes, stirred at 23 °C for 5 minutes, and then cooled again to 0 °C. Benzyl bromide (7.15 mL, 60.1 mmol, 1.5 equiv) was added, and the reaction mixture was stirred at 0 °C for 30 minutes and then partitioned between half-saturated NH<sub>4</sub>Cl (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 20 → 40% EtOAc in hexanes) provided trans-2*S*-benzyl-7-methyl-oct-4-enoic acid (2*R*-hydroxy-1*R*-methyl- 2-phenyl-ethyl)-methyl amide (12.0 g, 76%) as a viscous oil: *R<sub>f</sub>* = 0.54 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3382, 1617; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.81-0.90 (m), 1.42-1.61 (m), 1.80-1.95 (m), 2.17-2.25 (m), 2.33-2.54 (m), 2.55 (s), 2.73-2.99 (m), 3.05-3.16 (m), 3.93-4.00 (m), 4.31-4.51 (m), 5.25-5.56 (m), 7.14-7.37 (m); Anal. (C<sub>26</sub>H<sub>35</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 3*R*-Benzyl-5*S*-(1*R*-bromo-3-methyl-butyl)-dihydrofuran-2-one.**

[0087] *N*-Bromosuccinimide (5.97 g, 33.5 mmol, 1.1 equiv) was added in small portions over 5 minutes to a solution of trans-2*S*-benzyl-7-methyl-oct-4-enoic acid (2*R*-hydroxy- 1*R*-methyl-2-phenyl-ethyl)-methyl amide (12.0 g, 30.5 mmol, 1 equiv) and glacial acetic acid (8.73 mL, 152 mmol, 5.0 equiv) in a 4:1 mixture of THF and H<sub>2</sub>O (250 mL) at 0 °C. The resulting yellow solution was stirred for 15 minutes at 0 °C, then warmed to 23 °C, and subsequently refluxed for 1 hour.

After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (300 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (5% EtOAc in hexanes) gave 3*R*-benzyl-5*S*-(1*R*-bromo-3-methyl-butyl)-dihydrofuran-2-one (7.09 g, 65%) as a pale yellow oil: *R*<sub>f</sub> = 0.79 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1777; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.87 (d, 3H, *J* = 6.5), 0.94 (d, 3H, *J* = 6.9), 1.53-1.72 (m, 2H), 1.82-1.93 (m, 1H), 2.10-2.20 (m, 1H), 2.23-2.33 (m, 1H), 2.83 (dd, 1H, *J* = 13.5, 8.9), 3.04-3.12 (m, 1H), 3.14-3.22 (m, 1H), 4.05-4.12 (m, 1H), 4.23-4.29 (m, 1H), 7.20-7.36 (m, 5H); Anal. (C<sub>16</sub>H<sub>21</sub>BrO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 5*S*-(1*S*-Azido-3-methyl-butyl)-3*R*-benzyl-dihydrofuran-2-one.**

[0088] A suspension of sodium azide (2.83 g, 43.5 mmol, 2.0 equiv) and 3*R*-benzyl-5*S*-(1*R*-bromo-3-methyl-butyl)-dihydrofuran-2-one (7.09 g, 21.8 mmol, 1 equiv) in *N,N*-dimethylformamide (50 mL) was heated at 50 °C for 20 hours. The reaction mixture was cooled to 23 °C and partitioned between water (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (10% EtOAc in hexanes) to give 5*S*-(1*S*-azido-3-methyl-butyl)-3*R*-benzyl-dihydrofuran-2-one (3.26 g, 52%) as a colorless oil: *R*<sub>f</sub> = 0.47 (20% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2109, 1775; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.93 (d, 3H, *J* = 6.5), 0.94 (d, 3H, *J* = 6.5), 1.32-1.41 (m, 1H), 1.55-1.65 (m, 1H), 1.70-1.85 (m, 1H), 2.03-2.18 (m, 2H), 2.80 (dd, 1H, *J* = 13.5, 8.9), 3.05-3.22 (m, 2H), 3.27-3.33 (m, 1H), 4.22-4.27 (m, 1H), 7.18-7.36 (m, 5H); Anal. (C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**Preparation of Intermediate [1*S*-(4*R*-Benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-3-methyl-butyl]-carbamic Acid *tert*-Butyl Ester.**

[0089] A suspension of 5*S*-(1*S*-azido-3-methyl-butyl)-3*R*-benzyl-dihydrofuran-2-one (3.26 g, 11.3 mmol, 1 equiv) and Pd/C (10%, 0.40 g) in CH<sub>3</sub>OH (60 mL) was stirred under a hydrogen atmosphere (balloon) for 2 hours. The reaction mixture

was filtered through celite, concentrated, and the residue dissolved in 1,4-dioxane (80 mL). *N,N*-diisopropylethylamine (3.94 mL, 22.6 mmol, 2.0 equiv) and di-*tert*-butyl dicarbonate (3.70 g, 17.0 mmol, 1.5 equiv) were added sequentially, and the resulting solution was stirred at 23 °C for 2 hours. The reaction mixture was then partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution, 10 → 15% EtOAc in hexanes) provided [1*S*-(4*R*-benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-3-methyl-butyl]-carbamic acid *tert*-butyl ester (2.53 g, 62%) as a white solid: mp = 84-86 °C; *R*<sub>f</sub> = 0.66 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3338, 1767, 1704; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.89 (d, 3H, *J* = 6.5), 0.90 (d, 3H, *J* = 6.5), 1.18-1.32 (m, 1H), 1.40 (s, 9H), 1.43-1.56 (m, 1H), 1.98-2.07 (m, 1H), 2.20-2.29 (m, 1H), 2.78 (dd, 1H, *J* = 13.7, 9.0), 2.91-3.01 (m, 1H), 3.15 (dd, 1H, *J* = 13.7, 4.4), 3.71-3.81 (m, 1H), 4.23-4.28 (m, 1H), 4.34 (d, 1H, *J* = 9.7), 7.16-7.33 (m, 6H); Anal. (C<sub>21</sub>H<sub>31</sub>NO<sub>4</sub>) C, H, N.

#### **Preparation of Intermediate**

##### **Ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0090] Lithium hydroxide (7.2 mL of a 1 M aqueous solution, 7.2 mmol, 5.0 equiv) was added to a solution of [1*S*-(4*R*-benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-3-methyl-butyl]-carbamic acid *tert*-butyl ester (0.521 g, 1.44 mmol, 1 equiv) in DME (7 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 minutes and then partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). 4-Methylmorpholine *N*-oxide (0.337 g, 2.88 mmol, 2.0 equiv), powdered 4Å molecular sieves (0.55 g), and tetrapropylammonium perruthenate (0.050 g, 0.142 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 2.5 hours at 23 °C, and then it was filtered through celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (2.87 mmol, 2.0 equiv, prepared as described in Example 1 for the preparation of



ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate), 1-hydroxybenzotriazole hydrate (0.409 g, 3.03 mmol, 2.1 equiv), 4-methylmorpholine (0.633 mL, 5.76 mmol, 4.0 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.58 g, 3.03 mmol, 2.1 equiv) were added sequentially, and the reaction mixture was stirred for 15 hours at 23 °C and then partitioned between water (150 mL) and EtOAc (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 30 → 40% EtOAc in hexanes) provided ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.502 g, 44%) as a white foam: *R*<sub>f</sub> = 0.49 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1707, 1667; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.84-0.91 (m, 6H), 1.29 (t, 3H, *J* = 7.2); 1.39 (s, 9H), 1.42-1.61 (m, 4H), 1.98-2.05 (m, 1H), 2.35 (t, 2H, *J* = 7.2), 2.54 (d, 1H, *J* = 16.2), 2.70 (dd, 1H, *J* = 11.5, 5.6), 2.78-2.99 (m, 3H), 4.07-4.10 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.47 (s, br, 1H), 4.58 (d, 1H, *J* = 7.5), 5.46 (d, 1H, *J* = 15.8), 5.87 (d, 1H, *J* = 8.7), 6.58 (dd, 1H, *J* = 15.8, 5.1), 7.12-7.31 (m, 21H); Anal. (C<sub>49</sub>H<sub>59</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0091] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-Propenoate (0.096 g, 0.120 mmol, 1 equiv) in 1,4-dioxane (5 mL). The reaction mixture was stirred at 23 °C for 30 minutes and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and 4-methylmorpholine (0.033 mL, 0.300 mmol, 2.5 equiv) and benzyl chloroformate (0.025 mL, 0.175 mmol, 1.4 equiv) were added sequentially. The reaction mixture was stirred for 30 minutes at 23 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.050 g, 50%) as a white foam: *R*<sub>f</sub> = 0.38 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3318, 1712, 1664;

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.85-0.87 (m, 6H), 1.05-1.13 (m, 1H), 1.29 (t, 3H, *J* = 7.2); 1.35-1.42 (m, 2H), 1.97 (s, br, 1H), 2.29-2.31 (m, 2H), 2.54 (d, 1H, *J* = 16.5), 2.69-3.03 (m, 5H), 4.17 (q, 2H, *J* = 7.2), 4.47 (s, br, 1H), 4.81 (d, 1H, *J* = 7.5), 4.94-5.06 (m, 3H), 5.48 (d, 1H, *J* = 15.6), 5.87 (d, 1H, *J* = 8.1), 6.58 (dd, 1H, *J* = 15.6, 5.0), 7.12-7.32 (m, 26H); Anal. (C<sub>52</sub>H<sub>57</sub>N<sub>3</sub>O<sub>7</sub>•0.25 H<sub>2</sub>O) C, H, N.

**Preparation of Product Ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.**

[0092] Triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially to a solution of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.050 g, 0.060 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C, then carbon tetrachloride (4 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CBZ-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-propenoate (0.026 g, 73%) as a white solid: mp = 162-164 °C; *R*<sub>f</sub> = 0.66 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3412, 3292, 1718, 1689, 1650; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92 (d, 6H, *J* = 6.5), 1.30 (t, 3H, *J* = 7.2), 1.45-1.59 (m, 3H), 1.97-2.05 (m, 1H), 2.07 (d, 1H, *J* = 15.9), 2.17 (s, br, 2H), 2.70-2.79 (m, 1H), 2.91-3.09 (m, 3H), 4.18 (q, 2H, *J* = 7.2), 4.23-4.27 (m, 1H), 4.54 (s, br, 1H), 5.03 (d, 1H, *J* = 12.1), 5.08 (d, 1H, *J* = 12.1), 5.23 (d, 1H, *J* = 6.9), 5.38 (s, br, 1H), 5.47 (d, 1H, *J* = 15.6), 5.92 (d, 1H, *J* = 8.7), 6.43 (s, br, 1H), 6.60 (dd, 1H, *J* = 15.6, 4.8), 7.16-7.39 (m, 11H); Anal. (C<sub>33</sub>H<sub>43</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Example 3 - Preparation of Compound 2:**

**Ethyl-3-(EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate**

**Ethyl-3-[EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenoate.**

[0093] HCl (4 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.216 g, 0.269 mmol, 1 equiv) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6

mL), cooled to 0 °C, and *N,N*-diisopropylethylamine (0.094 mL, 0.540 mmol, 2.0 equiv) and ethyl chlorothioformate (0.034 mL, 0.326 mmol, 1.2 equiv) were added sequentially. The reaction mixture was stirred for 1 hour at 0 °C, and it then was partitioned between water (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was purified by flash column chromatography (40% EtOAc in hexanes) to afford ethyl-3-[EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.130 g, 61%) as a white foam: *R*<sub>f</sub> = 0.45 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1713, 1656; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.86 (d, 6H, *J* = 6.5), 1.05-1.19 (m, 1H), 1.21-1.39 (m, 8H), 1.41-1.58 (m, 2H), 1.96-2.05 (m, 1H), 2.28-2.35 (m, 2H), 2.54 (d, 1H, *J* = 14.6), 2.70 (dd, 1H, *J* = 11.7, 5.8), 2.79-3.00 (m, 4H), 4.17 (q, 2H, *J* = 7.2), 4.41-4.45 (m, 2H), 5.40 (d, 1H, *J* = 7.5), 5.49 (dd, 1H, *J* = 15.8, 1.6), 5.93 (d, 1H, *J* = 8.4), 6.59 (dd, 1H, *J* = 15.8, 5.1), 7.10-7.31 (m, 21H); Anal. (C<sub>47</sub>H<sub>55</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-Propenoate**

[0094] Ethyl-3-[EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.120 g, 0.152 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C, then carbon tetrachloride (6 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(EtSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-propenoate (0.056 g, 68%) as a beige foam: *R*<sub>f</sub> = 0.55 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3385, 2293, 3199, 1717, 1653; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.91 (d, 3H, *J* = 6.2), 0.92 (d, 3H, *J* = 6.5), 1.21-1.38 (m, 8H), 1.46-1.65 (m, 2H), 1.83-2.05 (m, 1H), 2.61 (d, 1H, *J* = 14.9), 2.74-3.09 (m, 7H), 4.18 (q, 2H, *J* = 7.2), 4.49-4.56 (m, 2H), 5.49 (d, 1H, *J* = 15.6), 5.59 (s, br, 1H), 6.05 (d, 1H, *J* = 8.7), 6.20 (d, 1H, *J* = 6.9), 6.49 (s, br, 1H), 6.62 (dd, 1H, *J* = 15.6, 4.8), 7.16-7.32 (m, 6H); Anal. (C<sub>28</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 4 - Preparation of Compound 6: Ethyl-3-(BnSCO-L-Leu Ψ [COCH<sub>2</sub>]-L-Phe- L-Gln)-E-Propenoate.**

**Preparation of Intermediate Benzyl Chlorothiolformate**

[0095] Triethylamine (7.12 mL, 51.1 mmol, 1.0 equiv) was added to a 0 °C solution of benzyl mercaptan (6.0 mL, 51.1 mmol, 1 equiv) and triphosgene (5.76 g, 19.4 mmol, 0.38 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The reaction mixture was warmed to 23 °C, stirred for 2 hours, and then concentrated under reduced pressure. The resulting white suspension was slurried with Et<sub>2</sub>O (100 mL) and filtered through a medium frit. The filtrate was concentrated under reduced pressure, and the resulting liquid was distilled under vacuum to provide benzyl chlorothiolformate (6.95 g, 73%) as a colorless liquid: bp = 95-100 °C (8 torr); IR (cm<sup>-1</sup>) 1755; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 4.19 (s, 2H), 7.30-7.34 (m, 5H).

**Preparation of Intermediate**

**Ethyl-3-[BnSCO-L-Leu □ [COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]- E-Propenoate.**

[0096] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-Leu □ [COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.190 g, 0.237 mmol, 1 equiv) in 1,4-dioxane (5 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), and 4-methylmorpholine (0.078 mL, 0.709 mmol, 3.0 equiv) and benzyl chlorothiolformate (0.050 mL, 0.331 mmol, 1.4 equiv) were added sequentially. The reaction mixture was stirred for 1.5 hours at 23 °C, and it then was partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[BnSCO-L-Leu Ψ [COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.117 g, 58%) as a white foam: R<sub>f</sub> = 0.44 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3312, 1714, 1656; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 0.82-0.89 (m, 6H), 1.28 (t, 3H, *J* = 7.2), 1.43-1.57 (m, 2H), 1.95-2.05 (m, 1H), 2.31-2.36 (m, 2H), 2.53 (d, 1H, *J* = 14.6), 2.56-2.70 (m, 1H), 2.72-3.04 (m, 5H), 4.02-4.21 (m, 4H), 4.44 (s, br, 2H), 5.41 (d, 1H, *J* = 7.5),

5.48 (dd, 1H,  $J = 15.8, 1.6$ ), 5.88 (d, 1H,  $J = 8.1$ ), 6.58 (dd, 1H,  $J = 15.8, 5.1$ ), 7.08-7.31 (m, 26H); Anal. ( $C_{52}H_{57}N_3O_6S$ ) C, H, N.

**Preparation of Product Ethyl-3-(BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate**

[0097] Ethyl-3-[BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.117 g, 0.137 mmol) was dissolved in  $CH_2Cl_2$  (5 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 20 minutes at 23 °C, then carbon tetrachloride (5 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5%  $CH_3OH$  in  $CH_2Cl_2$ ) to afford ethyl-3-(BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-propenoate (0.068 g, 81%) as a white foam:  $R_f = 0.52$  (10%  $CH_3OH$  in  $CH_2Cl_2$ ); IR ( $cm^{-1}$ ) 3299, 1717, 1650;  $^1H$  NMR ( $CDCl_3$ )  $\delta$  0.91 (d, 6H,  $J = 6.2$ ), 1.29 (t, 3H,  $J = 7.2$ ), 1.36-1.63 (m, 3H), 1.80 (s, br, 1H), 1.94-2.05 (m, 1H), 2.16-2.18 (m, 2H), 2.60 (d, 1H,  $J = 16.5$ ), 2.69-2.78 (m, 1H), 2.87-3.13 (m, 3H), 4.05-4.21 (m, 4H), 4.52 (s, br, 2H), 5.47 (d, 1H,  $J = 15.6$ ), 5.57 (s, 1H), 6.06 (d, 1H,  $J = 8.7$ ), 6.32 (d, 1H,  $J = 7.2$ ), 6.40 (s, 1H), 6.60 (dd, 1H,  $J = 15.6, 4.5$ ), 7.15-7.29 (m, 10H); Anal. ( $C_{33}H_{43}N_3O_6S$ ) C, H, N.

**Example 5 - Preparation of Compound 8:**

**Ethyl-3-(CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate Cyclopentyl Chlorothiolformate.**

[0098] Triethylamine (10.4 mL, 74.6 mmol, 1.0 equiv) was added to a 0 °C solution of cyclopentyl mercaptan (8.0 mL, 74.8 mmol, 1 equiv) and triphosgene (8.43 g, 28.4 mmol, 0.38 equiv) in  $CH_2Cl_2$ . The reaction mixture was warmed to 23 °C, stirred for 2 hours, and then concentrated under reduced pressure. The resulting white suspension was slurried with  $Et_2O$  (100 mL) and filtered through a medium frit. The filtrate was concentrated under reduced pressure, and the resulting liquid was distilled under vacuum to provide cyclopentyl chlorothiolformate (10.4 g, 85%) as a colorless liquid: bp = 70-74 °C (1 torr); IR

(cm<sup>-1</sup>) 1756, 830; <sup>1</sup>H NMR (C<sub>6</sub>D<sub>6</sub>) δ 1.01-1.23 (m, 6H), 1.49-1.60 (m, 2H), 3.20-3.29 (m, 1H).

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenoate**

[0099] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.205 g, 0.256 mmol, 1 equiv) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), cooled to 0 °C, and 4-methylmorpholine (0.070 mL, 0.637 mmol, 2.5 equiv) and cyclopentyl chlorothiolformate (0.063 mL, 0.383 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 30 minutes at 0 °C, and it then was partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to provide ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.113 g, 53%) as a white foam: *R*<sub>f</sub> = 0.44 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3310, 1713, 1654; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.85 (d, 6H, *J* = 6.5), 1.02-1.12 (m, 1H), 1.29 (t, 3H, *J* = 7.2), 1.42-1.68 (m, 7H), 1.98-2.08 (m, 4H), 2.31-2.35 (m, 2H), 2.55 (d, 1H, *J* = 14.3), 2.70 (dd, 1H, *J* = 11.8, 5.9), 2.79-3.09 (m, 4H), 3.55-3.66 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.38 (s, br, 1H), 4.48-4.49 (m, 1H), 5.33 (d, 1H, *J* = 7.5), 5.50 (dd, 1H, *J* = 15.9, 1.6), 5.94 (d, 1H, *J* = 8.4), 6.60 (dd, 1H, *J* = 15.9, 5.0), 7.10-7.31 (m, 21H); Anal. (C<sub>50</sub>H<sub>59</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate**

[0100] Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.090 g, 0.108 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 20 minutes at 23 °C, then carbon tetrachloride (4 mL) was added, and the mixture was concentrated

under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to provide ethyl-3-(CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-propenoate (0.050 g, 79%) as a white foam: *R*<sub>f</sub> = 0.58 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3385, 3298, 3199, 1717, 1652; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.89-0.93 (m, 6H), 1.31 (t, 3H, *J* = 7.2), 1.40-1.69 (m, 8H), 1.86 (s, br, 1H), 2.04-2.09 (m, 4H), 2.21 (s, br, 2H), 2.58 (d, 1H, *J* = 9.3), 2.64-2.79 (m, 1H), 2.89-3.07 (m, 3H), 3.07-3.69 (m, 1H), 4.18 (q, 2H, *J* = 7.2), 4.47 (s, br, 1H), 4.56 (s, br, 1H), 5.49 (d, 1H, *J* = 15.2), 5.57 (s, br, 1H), 6.07 (d, 1H, *J* = 8.7), 6.15 (d, 1H, *J* = 6.9), 6.54 (s, br, 1H), 6.62 (dd, 1H, *J* = 15.2, 4.8), 7.16-7.33 (m, 5H); Anal. (C<sub>31</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 6 - Preparation of Compound 4:**  
**Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe- L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate trans-6-Methyl-hept-4-enoic Acid**

[0101] A solution of isobutyraldehyde (9.59 g, 133 mmol, 1 equiv) in THF (50 mL) was added dropwise via addition funnel to a solution of vinylmagnesium bromide (133 mL of a 1.0 M solution in THF, 133 mmol, 1.0 equiv) in THF (300 mL) at 0 °C. Upon completion of the addition, the reaction mixture was stirred for 30 minutes at 0 °C, then ethyl malonyl chloride (17.0 mL, 133 mmol, 1.0 equiv) was added. After stirring for 1 hour at 0°C, the reaction mixture was partitioned between saturated NH<sub>4</sub>Cl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by filtration through silica gel (eluting with 5% EtOAc in hexanes) afforded the intermediate malonate ester (11.5 g, 40% yield). This material was not characterized, but was combined (neat) with Ti(OEt)<sub>4</sub> (1.13 mL, 5.39 mmol, 0.10 equiv), heated to 190 °C for 4 hours, and then cooled to 60 °C. EtOH (50 mL) and 6.0 M KOH (50 mL) were added sequentially, and the brown reaction mixture was refluxed for 4 hours. After cooling to 23 °C, the reaction mixture was filtered through a medium frit, and the filtrate was partitioned between water (150 mL) and Et<sub>2</sub>O (2 x 150 mL). The aqueous layer was then acidified to pH = 2 (as indicated by pH paper) with concentrated HCl and extracted with a 1:1

mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was distilled at reduced pressure to afford trans-6-methyl-hept-4-enoic acid (3.58 g, 47%) as a colorless liquid: bp: 107-112 °C (1 torr); IR (cm<sup>-1</sup>) 2960, 1711; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.96 (d, 6H, *J* = 6.5) 2,18-2.45 (m, 5H), 5.31-5.50 (m, 2H); Anal. (C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>) C, H.

**Preparation of Intermediate trans-6-Methyl-hept-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0102] Oxalyl chloride (2.25 mL, 25.8 mmol, 1.05 equiv) was added to a solution of trans-6-methyl-hept-4-enoic acid (3.50 g, 24.6 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.016 equiv) in benzene (60 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 2 hours and then concentrated under reduced pressure. The resulting oil was dissolved in THF (20 mL) and added via cannula to a solution of 1*R*,2*R*-(-)-pseudoephedrine (3.87 g, 23.4 mmol, 1.0 equiv) and triethylamine (3.92 mL, 28.1 mmol, 1.2 equiv) in THF (150 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 minutes and then partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and EtOAc (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (gradient elution 40 → 50% EtOAc in hexanes) to afford trans-6-methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.31 g, 93%) as a viscous oil: *R*<sub>f</sub> = 0.35 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3382, 1622; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.96 (d, *J* = 6.8), 0.97 (d, *J* = 6.5), 1.11 (d, *J* = 6.9), 2.18-2.59 (m), 2.82 (s), 2.92 (s), 3.99-4.04 (m), 4.32-4.42 (m), 4.44-4.49 (m), 4.55-4.62 (m), 5.32-5.49 (m), 7.24-7.42 (m); Anal. (C<sub>18</sub>H<sub>27</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate trans-2*S*-Benzyl-6-methyl-hept-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0103] *n*-Butyllithium (28.6 mL of a 1.6 M solution in hexanes, 45.8 mmol, 2.1 equiv) was added to a suspension of anhydrous lithium chloride (6.47 g, 153 mmol, 7.0 equiv) and diisopropylamine (6.88 mL, 49.1 mmol, 2.25 equiv) in THF



(250 mL) at -78 °C. The reaction mixture was stirred for 20 minutes at -78 °C, and it then was maintained at 0 °C for 5 minutes and subsequently cooled again to -78 °C. *trans*-6-Methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.31 g, 21.8 mmol, 1 equiv) in THF (40 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1 hour, maintained at 0 °C for 15 minutes, stirred at 23 °C for 5 minutes, and then cooled again to 0 °C. Benzyl bromide (3.89 mL, 32.7 mmol, 1.5 equiv) was added, the reaction mixture was stirred at 0 °C for 30 minutes, and it then was partitioned between half-saturated NH<sub>4</sub>Cl (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 20 → 40% EtOAc in hexanes) provided *trans*-2*S*-benzyl-6-methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (7.91 g, 96%) as a viscous oil: *R*<sub>f</sub> = 0.52 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3383, 1616; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.82-0.91 (m), 0.96 (d, *J* = 6.5), 1.24-1.27 (m), 2.14-2.47 (m), 2.56 (s), 2.72-2.99 (m), 3.04-3.15 (m), 3.93-4.00 (m), 4.31-4.51 (m), 5.21-5.39 (m), 5.42-5.55 (m), 7.14-7.37 (m); Anal. (C<sub>25</sub>H<sub>33</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 3*R*-Benzyl-5*S*-(1*R*-bromo-2-methyl-propyl)-dihydrofuran-2-one.**

[0104] *N*-Bromosuccinimide (3.89 g, 21.9 mmol, 1.05 equiv) was added in small portions over 5 minutes to a solution of *trans*-2*S*-benzyl-6-methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (7.90 g, 20.8 mmol, 1 equiv) and glacial acetic acid (5.96 mL, 104 mmol, 5.0 equiv) in a 4:1 mixture of THF and H<sub>2</sub>O (250 mL) at 0 °C. The resulting yellow solution was stirred for 15 minutes at 0 °C, then was warmed to 23 °C, and subsequently was refluxed for 1 hour. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (300 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (5% EtOAc in hexanes) gave

3*R*-benzyl-5*S*-(1*R*-bromo-2-methyl-propyl)- dihydrofuran-2-one (5.09 g, 79%) as a white solid. Minor impurities were removed by recrystallization from hexanes (2.51 g recovery): mp = 75-76 °C;  $R_f$  = 0.64 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1774; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.93 (d, 3H,  $J$  = 6.6), 0.99 (d, 3H,  $J$  = 6.9), 2.05-2.18 (m, 1H), 2.20-2.33 (m, 2H), 2.83 (dd, 1H,  $J$  = 13.6, 8.7), 2.95-3.05 (m, 1H), 3.17 (dd, 1H,  $J$  = 13.6, 4.5), 3.89 (dd, 1H,  $J$  = 9.0, 3.4), 4.32-4.39 (m, 1H), 7.20-7.36 (m, 5H); Anal. (C<sub>15</sub>H<sub>19</sub>BrO<sub>2</sub>) C, H.

**Preparation of Intermediate 5*S*-(1*S*-Azido-2-methyl-propyl)-3*R*-benzyl-dihydrofuran-2-one.**

[0105] A solution of Aliquat-336 (0.163 g, 0.403 mmol, 0.05 equiv) and 3*R*-benzyl-5*S*-(1*R*-bromo-2-methyl-propyl)-dihydrofuran-2-one (2.51 g, 8.06 mmol, 1 equiv) in toluene (60 mL) was treated with a solution of sodium azide (2.10 g, 32.3 mmol, 4.0 equiv) in H<sub>2</sub>O (10 mL). The resulting biphasic mixture was heated to 70 °C and maintained at that temperature for 48 hours. The reaction mixture was cooled to 23 °C and partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (gradient elution, 5 → 10% EtOAc in hexanes) to give 5*S*-(1*S*-azido-2-methyl-propyl)- 3*R*-benzyl-dihydrofuran-2-one (1.0 g, 45%) as a viscous oil:  $R_f$  = 0.41 (20% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2105, 1772; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.86-1.04 (m, 7H), 1.95-2.17 (m, 2H), 2.83 (dd, 1H,  $J$  = 13.2, 8.3), 2.92 (dd, 1H,  $J$  = 6.4, 4.5), 3.05-3.21 (m, 2H), 4.33-4.38 (m, 1H), 7.19-7.35 (m, 5H); Anal. (C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**Preparation of Intermediate [1*S*-(4*R*-Benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-2-methyl-propyl]-carbamic Acid *tert*-Butyl Ester.**

[0106] A suspension of 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-benzyl-dihydrofuran-2-one (1.00 g, 3.66 mmol, 1 equiv) and Pd/C (10%, 0.090 g) in CH<sub>3</sub>OH (60 mL) was stirred under a hydrogen atmosphere (balloon) for 1 hour. The reaction mixture was filtered through celite, concentrated, and the residue

dissolved in 1,4-dioxane (50 mL). *N,N*-diisopropylethylamine (1.28 mL, 7.35 mmol, 2.0 equiv) and di-*tert*-butyl dicarbonate (1.20 g, 5.50 mmol, 1.5 equiv) were added sequentially, and the resulting solution was stirred at 23 °C for 18 hours. The reaction mixture was then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution, 10 → 15% EtOAc in hexanes) provided [1*S*-(4*R*-benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-2-methyl-propyl]- carbamic acid *tert*-butyl ester (0.496 g, 39%) as a colorless oil: *R*<sub>f</sub> = 0.44 (20% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3340, 1768, 1708; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92 (d, 6H, *J* = 6.9), 1.41 (s, 9H), 1.70-1.80 (m, 1H), 1.98-2.07 (m, 1H), 2.14-2.24 (m, 1H), 2.81 (dd, 1H, *J* = 13.6, 8.9), 2.92-3.01 (m, 1H), 3.13 (dd, 1H, *J* = 13.6, 4.4), 3.31-3.38 (m, 1H), 4.39-4.47 (m, 2H), 7.17-7.33 (m, 5H); Anal. (C<sub>20</sub>H<sub>29</sub>NO<sub>4</sub>) C, H, N.

#### **Preparation of Intermediate**

##### **Ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]- E-Propenoate.**

[0107] Lithium hydroxide (7.14 mL of a 1 M aqueous solution, 7.14 mmol, 5.0 equiv) was added to a solution of [1*S*-(4*R*-benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-2-methyl-propyl]- carbamic acid *tert*-butyl ester (0.496 g, 1.43 mmol, 1 equiv) in DME (7 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 minutes and then partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). 4-Methylmorpholine *N*-oxide (0.334 g, 2.85 mmol, 2.0 equiv), powdered 4Å molecular sieves (0.51 g), and tetrapropylammonium perruthenate (0.050 g, 0.142 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 2.5 hours at 23 °C and then filtered through celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl, prepared as described in Example 1 for the preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate (1.86 mmol,

1.3 equiv), 1-hydroxybenzotriazole hydrate (0.289 g, 2.14 mmol, 1.5 equiv), 4-methylmorpholine (0.629 mL, 5.72 mmol, 4.0 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.411 g, 2.14 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 8 hours at 23 °C then partitioned between water (150 mL) and EtOAc (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided

ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.497 g, 44%) as an off-white foam:  $R_f$  = 0.40 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1709, 1663; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.65 (d, 3H,  $J$  = 6.9), 0.91 (d, 1H,  $J$  = 6.9), 1.29 (t, 3H,  $J$  = 7.2), 1.40 (s, 9H), 1.94-2.20 (m, 2H), 2.30-2.35 (m, 2H), 2.51 (d, 1H,  $J$  = 17.1), 2.68 (dd, 1H,  $J$  = 11.7, 5.8), 2.74-2.90 (m, 3H), 3.04 (dd, 1H,  $J$  = 17.3, 9.8), 4.05-4.09 (m, 1H), 4.17 (q, 2H,  $J$  = 7.2), 4.38-4.45 (m, 1H), 4.83 (d, 1H,  $J$  = 8.1), 5.45 (d, 1H,  $J$  = 15.7), 5.87 (d, 1H,  $J$  = 8.1), 6.57 (dd, 1H,  $J$  = 15.7, 5.0), 7.10-7.31 (m, 21H); Anal. (C<sub>48</sub>H<sub>57</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

#### **Preparation of Intermediate**

##### **Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]- E-Propenoate.**

[0108] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.225 g, 0.286 mmol, 1 equiv) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and *N,N*-diisopropylethylamine (0.125 mL, 0.718 mmol, 2.5 equiv) and ethyl chlorothioformate (0.040 mL, 0.384 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 1 hour at 23 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]- E-propenoate (0.148 g, 66%) as a white foam:  $R_f$  = 0.37 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1714,

1653;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.68 (d, 3H,  $J = 6.9$ ), 0.92 (d, 3H,  $J = 6.5$ ), 1.21-1.32 (m, 7H), 1.92-2.03 (m, 2H), 2.32-2.36 (m, 2H), 2.51 (dd, 1H,  $J = 17.4, 2.2$ ), 2.67 (dd, 1H,  $J = 11.8, 5.9$ ), 2.73-2.90 (m, 4H), 3.05 (dd, 1H,  $J = 17.6, 9.8$ ), 4.17 (q, 2H,  $J = 7.2$ ), 4.39-4.44 (m, 2H), 5.46 (dd, 1H,  $J = 15.7, 1.7$ ), 5.62 (d, 1H,  $J = 8.1$ ), 5.96 (d, 1H,  $J = 8.4$ ), 6.57 (dd, 1H,  $J = 15.7, 5.1$ ), 7.10-7.12 (m, 2H), 7.15-7.31 (m, 19H); Anal. ( $\text{C}_{46}\text{H}_{53}\text{N}_3\text{O}_6\text{S}$ ) C, H, N.

#### Preparation of Product

##### Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.

[0109] Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.148 g, 0.191 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 20 minutes at 23 °C, then carbon tetrachloride (6 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5%  $\text{CH}_3\text{OH}$  in  $\text{CH}_2\text{Cl}_2$ ) to afford ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-

L-Phe-L-Gln)-E-propenoate (0.078 g, 77%) as a white solid: mp = 205 °C (dec);  $R_f$  = 0.45 (10%  $\text{CH}_3\text{OH}$  in  $\text{CH}_2\text{Cl}_2$ ); IR ( $\text{cm}^{-1}$ ) 3424, 3304, 1715, 1658, 1640, 1624;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  0.75 (d, 3H,  $J = 6.5$ ), 0.82 (d, 3H,  $J = 6.9$ ), 1.14 (t, 3H,  $J = 7.2$ ), 1.21 (t, 3H,  $J = 7.2$ ), 1.56-1.71 (m, 2H), 1.99-2.11 (m, 2H), 2.44-2.59 (m, 4H), 2.70-2.85 (m, 3H), 2.93-2.95 (m, 1H), 4.06-4.18 (m, 3H), 4.31 (s, br, 1H), 5.53 (d, 1H,  $J = 15.6$ ), 6.63 (dd, 1H,  $J = 15.6, 5.1$ ), 6.75 (s, 1H), 7.14-7.26 (m, 6H), 8.06 (d, 1H,  $J = 8.1$ ), 8.33 (d, 1H,  $J = 8.1$ ); Anal. ( $\text{C}_{27}\text{H}_{39}\text{N}_3\text{O}_6\text{S}$ ) C, H, N.

#### Example 7 - Preparation of Compound 5:

##### Ethyl-3-(BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate

#### Preparation of Intermediate

##### Ethyl-3-[BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenoate.

[0110] HCl (8 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.272 g, 0.345 mmol, 1 equiv) in 1,4-dioxane (10 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in  $\text{CH}_2\text{Cl}_2$  (10

mL), and 4-methylmorpholine (0.095 mL, 0.864 mmol, 2.5 equiv) and benzyl chlorothiolformate (0.068 mL, 0.450 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 1 hour at 23 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.160 g, 55%) as a white foam: *R*<sub>f</sub> = 0.41 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3316, 1716, 1658; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.66 (d, 3H, *J* = 6.9), 0.92 (d, 3H, *J* = 6.5), 1.29 (t, 3H, *J* = 7.2), 1.93-2.03 (m, 2H), 2.32-2.36 (m, 2H), 2.50 (dd, 1H, *J* = 17.7, 2.5), 2.67 (dd, 1H, *J* = 11.5, 6.2), 2.73-2.89 (m, 3H), 3.07 (dd, 1H, *J* = 17.7, 10.0), 4.09 (s, 2H), 4.16 (q, 2H, *J* = 7.2), 4.38-4.42 (m, 2H), 5.46 (dd, 1H, *J* = 15.8, 1.6), 5.65 (d, 1H, *J* = 8.4), 5.95 (d, 1H, *J* = 8.1), 6.57 (dd, 1H, *J* = 15.8, 5.1), 7.03 (s, 1H), 7.09-7.12 (m, 2H), 7.15-7.31 (m, 23H); Anal. (C<sub>51</sub>H<sub>55</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

#### **Preparation of Product**

##### **Ethyl-3-(BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-Propenoate.**

[0111] Ethyl-3-[BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.160 g, 0.191 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 20 minutes at 23 °C, then carbon tetrachloride (5 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to provide ethyl-3-(BnSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-propenoate (0.097 g, 85%) as a white solid: mp = 185-189 °C; *R*<sub>f</sub> = 0.55 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3392, 3287, 1710, 1643; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.74 (d, 3H, *J* = 6.9), 0.82 (d, 3H, *J* = 6.5), 1.21 (t, 3H, *J* = 7.2), 1.62-1.70 (m, 2H), 1.98-2.12 (m, 3H), 2.44-2.59 (m, 2H), 2.71-2.96 (m, 3H), 4.03 (s, 2H), 4.10 (q, 2H, *J* = 7.2), 4.19-4.23 (m, 1H), 4.32 (s, br, 1H), 5.54 (d, 1H, *J* = 15.6), 6.64 (dd, 1H, *J* = 15.6, 5.3), 6.75 (s, 1H), 7.14-7.28 (m, 11H), 8.06 (d, 1H, *J* = 8.1), 8.42 (d, 1H, *J* = 7.8); Anal. (C<sub>32</sub>H<sub>41</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 8 - Preparation of Compound 7: Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenoate**

[0112] HCl (6 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.248 g, 0.315 mmol, 1 equiv) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 23 °C for 1.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL), cooled to 0 °C, and 4-methylmorpholine (0.086 mL, 0.782 mmol, 2.5 equiv) and cyclopentyl chlorothiolformate (0.067 mL, 0.407 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 30 minutes at 0 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to provide ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.126 g, 49%) as a white foam: *R*<sub>f</sub> = 0.42 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1711, 1654; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.67 (d, 3H, *J* = 6.9), 0.92 (d, 3H, *J* = 6.9), 1.29 (t, 3H, *J* = 7.2), 1.45-1.68 (m, 6H), 1.91-2.05 (m, 4H), 2.31-2.34 (m, 2H), 2.51 (d, 1H, *J* = 17.4), 2.67 (dd, 1H, *J* = 11.5, 6.2), 2.75-2.90 (m, 3H), 3.03 (dd, 1H, *J* = 17.3, 9.8), 3.60-3.64 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.36-4.44 (m, 2H), 5.46 (dd, 1H, *J* = 15.8, 1.7), 5.57 (d, 1H, *J* = 8.1), 5.95 (d, 1H, *J* = 8.4), 6.58 (dd, 1H, *J* = 15.8, 5.0), 7.10-7.12 (m, 2H), 7.19-7.31 (m, 19H); Anal. (C<sub>49</sub>H<sub>57</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-Gln)-E-Propenoate**

[0113] Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate (0.126 g, 0.154 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), and triisopropylsilane (0.10 mL) and trifluoroacetic acid (6 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 20 minutes at 23 °C, then carbon tetrachloride (6 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column

chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-propenoate (0.046 g, 52%) as a white solid: mp = 200-204 °C; R<sub>f</sub> = 0.38 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3431, 3261, 1717, 1642; <sup>1</sup>H NGR (DMSO-*d*<sub>6</sub>) δ 0.75 (d, 3H, *J* = 6.5), 0.82 (d, 3H, *J* = 6.5), 1.22 (t, 3H, *J* = 7.2), 1.40-1.74 (m, 7H), 1.95-2.10 (m, 6H), 2.43-2.59 (m, 2H), 2.71-2.84 (m, 2H), 2.93-2.95 (m, 1H), 3.47-3.56 (m, 1H), 4.06-4.16 (m, 3H), 4.32 (s, br, 1H), 5.54 (d, 1H, *J* = 15.9), 6.64 (dd, 1H, *J* = 15.9, 5.3), 6.75 (s, 1H), 7.15-7.27 (m, 6H), 8.06 (d, 1H, *J* = 7.8), 8.27 (d, 1H, *J* = 8.1); Anal. (C<sub>30</sub>H<sub>43</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 9 - Preparation of Compound 3: (2,3-Dihydroindole)-3-(BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-Propenamide.**

**Preparation of Intermediate**

**[2-(2,3-Dihydro-indol-1-yl)-2-oxo-ethyl]-phosphonic Acid Diethyl Ester.**

[0114] Oxalyl chloride (5.96 mL, 68.3 mmol, 1.05 equiv) was added to a solution of diethylphosphonoacetic acid (12.8 g, 65.0 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.006 equiv) in benzene (150 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 1 hour and then concentrated under reduced pressure. The resulting oil was dissolved in THF (30 mL) and added via cannula to a solution of indoline (7.38 g, 61.9 mmol, 0.95 equiv) and triethylamine (10.9 mL, 78.0 mmol, 1.2 equiv) in THF (200 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 minutes, and it then was partitioned between 0.5 M HCl (150 mL) and EtOAc (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to afford a tan solid. Recrystallization from Et<sub>2</sub>O provided [2-(2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-phosphonic acid diethyl ester (12.2 g, 63%) as a light brown solid: mp: 97-99 °C; R<sub>f</sub> = 0.06 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3460, 1657, 1597, 1482; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 1.35 (t, 6H, *J* = 7.2), 3.14 (d, 2H, *J* = 22.4), 3.22 (d, 2H, *J* = 8.4), 4.15-4.30 (m, 6H), 7.04 (t, 1H, *J* = 7.0), 7.17-7.28 (m, 2H), 8.21 (d, 1H, *J* = 9.0); Anal. (C<sub>14</sub>H<sub>20</sub>NO<sub>4</sub>P) C, H, N.



**Preparation of Intermediate (2,3-Dihydroindole)-3-[BOC-L-(Tr-Gln)]-E-Propenamide.**

[0115] Sodium bis(trimethylsilyl)amide (11.9 mL of a 1.0 M solution in THF, 11.9 mmol, 1.0 equiv) was added to a solution of [2-(2,3-dihydro-indol-1-yl)-2-oxo-ethyl]-phosphonic acid diethyl ester (3.54 g, 11.9 mmol, 1.0 equiv) in THF (150 mL) at -78°C, and the resulting solution was stirred for 20 minutes at that temperature. Crude [BOC-L-(Tr-Gln)]-H (5.63 g, 11.9 mmol, 1 equiv), prepared as described in Example 1, in THF (40 mL) was added via cannula, and the reaction mixture was stirred for 1 hour at -78°C, warmed to 0 °C for 10 minutes, and partitioned between 0.5 M HCl (150 mL) and EtOAc (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (50% EtOAc in hexanes) provided (2,3-dihydroindole)-3-[BOC-L-(Tr-Gln)]-E-propenamide (6.35 g, 87%) as an off-white foam:  $R_f$  = 0.28 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3401, 3307, 1690, 1665; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.44 (s, 9H), 1.76-2.05 (m, 2H), 2.37-4.06 (m, 2H), 3.11-3.22 (m, 2H), 4.02-4.16 (m, 2H), 4.27-4.40 (m, 1H), 4.91-4.97 (m, 1H), 6.29 (d, 1H,  $J$  = 14.9), 6.77-6.96 (m, 2H), 6.98-7.05 (m, 1H), 7.14-7.37 (m, 17H), 8.25 (d, 1H,  $J$  = 7.5); Anal. (C<sub>39</sub>H<sub>41</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**Preparation of Intermediate (2,3-Dihydroindole)-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenamide.**

[0116] Lithium hydroxide (8.0 mL of a 1 M aqueous solution, 8.0 mmol, 5.0 equiv) was added to a solution of [1*S*-(4*R*-benzyl-5-oxo-tetrahydrofuran-2*S*-yl)-3-methyl-butyl]- carbamic acid *tert*-butyl ester (0.576 g, 1.59 mmol, 1 equiv), prepared as described in Example 1, in DME (8 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 45 minutes, and it then was partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). 4-Methylmorpholine *N*-oxide (0.373 g, 3.18 mmol, 2.0 equiv), powdered 4Å molecular sieves (0.60 g), and tetrapropylammonium perruthenate (0.056 g, 0.159

mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1 hour at 23°C and then filtered through celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). Crude (2,3-dihydroindole)-3-(H<sub>2</sub>N-L-Tr-*Gln*)-E-propenamide•HCl (prepared from (2,3-dihydroindole)-3-[BOC-L-(Tr-*Gln*)]-E-propenamide in a manner analogous to the method described in Example 1 for the preparation of ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (2.39 mmol, 1.5 equiv), 1-hydroxybenzotriazole hydrate (0.322 g, 2.38 mmol, 1.5 equiv), 4-methylmorpholine (0.699 mL, 6.36 mmol, 4.0 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.322 g, 2.38 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 16 hours at 23°C and then partitioned between water (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% hexanes in EtOAc) provided (2,3-dihydroindole)-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenamide (0.568 g, 40%) as tan foam: *R*<sub>f</sub> = 0.64 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3304, 1700, 1665; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.86 (d, 6H, *J* = 6.5), 0.97-1.12 (m, 1H), 1.39 (s, 9H), 1.45-1.64 (m, 2H), 2.39-2.43 (m, 2H), 2.51 (d, 1H, *J* = 15.6), 2.67 (dd, 1H, *J* = 12.3, 7.0), 2.79-2.96 (m, 3H), 3.16 (s, br, 2H), 4.01-4.20 (m, 5H), 4.52 (s, br, 1H), 4.63 (d, 1H, *J* = 7.8), 6.09 (d, 1H, *J* = 14.7), 6.17 (d, 1H, *J* = 8.1), 6.65 (dd, 1H, *J* = 14.7, 5.8), 6.99-7.04 (m, 1H), 7.11-7.39 (m, 23H), 8.26 (d, 1H, *J* = 7.5).

**Preparation of Product (2,3-Dihydroindole)-3-[BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-Propenamide**

[0117] HCl (6 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of (2,3-dihydroindole)-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenamide (0.204 g, 0.233 mmol, 1 equiv) in 1,4-dioxane (8 mL). The reaction mixture was stirred at 23 °C for 2.5 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 mL), and *N,N*-diisopropylethylamine (0.081 mL, 0.465 mmol, 2.0 equiv) and benzyl chlorothioformate (0.042 mL, 0.279 mmol, 1.2 equiv) were added sequentially. The reaction mixture was stirred for 2 hours at

23°C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to provide crude (2,3-dihydroindole)-3- [BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenamide as an off-white foam. This material was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and triisopropylsilane (0.075 mL), and trifluoroacetic acid (5 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C, then carbon tetrachloride (4 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford (2,3-dihydroindole)-3- (BnSCO-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-propenamide (0.016 g, 10%) as a white foam: *R*<sub>f</sub> = 0.51 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92 (d, 6H, *J* = 6.2), 1.11-1.25 (m, 1H), 1.33-1.36 (m, 1H), 1.49-2.17 (m, 4H), 2.25 (s, br, 2H), 2.58 (d, 1H, *J* = 17.1), 2.70-2.72 (m, 1H), 2.94-3.17 (m, 4H), 4.01-4.04 (m, 2H), 4.11 (s, 2H), 4.52-4.58 (m, 2H), 5.47 (s, br, 1H), 6.13 (d, 1H, *J* = 13.4), 6.29 (s, br, 1H), 6.41 (s, br, 1H), 6.66 (s, br, 1H), 7.00-7.05 (m, 1H), 7.17-7.29 (m, 13H), 8.25 (d, 1H, *J* = 7.2).

#### **Example 10 - Preparation of Compound 10:**

##### **Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]- L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

##### **Preparation of Intermediate trans-6-Methyl-2*S*-(4-methyl-benzyl)-hept-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0118] *n*-Butyllithium (33.7 mL of a 1.6 M solution in hexanes, 53.9 mmol, 2.15 equiv) was added to a suspension of anhydrous lithium chloride (7.47 g, 176 mmol, 7.0 equiv) and diisopropylamine (8.09 mL, 57.7 mmol, 2.3 equiv) in THF (260 mL) at -78 °C. The reaction mixture was stirred for 30 minutes at -78°C, maintained at 0 °C for 5 minutes, and subsequently cooled again to -78 °C. trans-6-Methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*- methyl-2-phenyl-ethyl)-methyl amide (7.26 g, 25.1 mmol, 1 equiv) in THF (50 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1.75 hours, maintained at 0 °C for 20 minutes, stirred at 23 °C for 5 minutes, and then cooled again to 0 °C. A

solution of 4-methylbenzyl bromide (6.96 g, 37.6 mmol, 1.5 equiv) in THF (15 mL) was added, and the reaction mixture was stirred at 0 °C for 30 minutes and then partitioned between half-saturated NH<sub>4</sub>Cl (230 mL) and a 1:1 mixture of EtOAc and hexanes (200 mL, 2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 20 → 40% EtOAc in hexanes) provided trans-6-methyl-2*S*-(4-methyl-benzyl)-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (9.33 g, 95%) as a viscous oil: *R*<sub>f</sub> = 0.44 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3378, 1619; <sup>1</sup>H NGR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.84-0.97 (m), 2.12-2.27 (m), 2.29 (s), 2.35-2.46 (m), 2.58 (s), 2.66-2.84 (m), 2.86-3.05 (m), 3.91-4.13 (m), 4.30-4.44 (m), 4.45-4.53 (m), 5.17-5.54 (m), 7.04 (s), 7.12-7.33 (m); Anal. (C<sub>26</sub>H<sub>35</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 5*S*-(1*R*-Bromo-2-methyl-propyl)-3*R*-(4-methyl-benzyl)-dihydrofuran-2-one**

[0119] *N*-Bromosuccinimide (4.37 g, 24.6 mmol, 1.05 equiv) was added in small portions over 10 minutes to a solution of trans-6-methyl-2*S*-(4-methyl-benzyl)-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (9.21 g, 23.4 mmol, 1 equiv) and glacial acetic acid (6.70 mL, 117 mmol, 5.0 equiv) in a 4:1 mixture of THF and H<sub>2</sub>O (250 mL) at 0 °C. The resulting yellow solution was stirred for 15 minutes at 0 °C, then warmed to 23 °C, and subsequently refluxed for 45 minutes. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL, 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (gradient elution 4 → 6% EtOAc in hexanes) gave 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-methyl-benzyl)-dihydrofuran-2-one (6.45 g, 85%) as a white solid (containing approximately 5-10% unidentified impurities by <sup>1</sup>H NGR): mp = 94-100 °C; *R*<sub>f</sub> = 0.64 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1772; <sup>1</sup>H NGR (CDCl<sub>3</sub>, major isomer) δ 0.93 (d, 3H, *J* = 6.5), 0.99 (d, 3H, *J* = 6.5), 2.05-2.18 (m, 1H), 2.21-2.28 (m, 2H), 2.33 (s, 3H), 2.79 (dd, 1H, *J* = 13.5, 9.0), 2.92-3.03 (m, 1H), 3.12 (dd, 1H, *J* =

13.5, 4.5), 3.89 (dd, 1H,  $J = 9.0, 3.4$ ), 4.32-4.41 (m, 1H), 7.06-7.16 (m, 4H); Anal. ( $C_{16}H_{21}BrO_2$ ) C, H.

**Preparation of Intermediate 5*S*-(1*S*-Azido-2-methyl-propyl)-3*R*-(4-methyl-benzyl)- dihydrofuran-2-one**

[0120] A suspension of sodium azide (2.55 g, 39.2 mmol, 2.0 equiv) and 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-methyl-benzyl)-dihydrofuran-2-one (6.37 g, 19.6 mmol, 1 equiv) in *N,N*-dimethylformamide (63 mL) was heated at 50 °C for 48 hours. The reaction mixture was cooled to 23 °C and partitioned between water (370 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 370 mL, 200 mL). The combined organic layers were dried over  $Na_2SO_4$ , concentrated, and the residue purified by flash column chromatography (gradient elution 8 → 12% EtOAc in hexanes) to give 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-methyl-benzyl)-dihydrofuran-2-one (3.33 g, 59%) as a colorless oil (containing approximately 5-10% unidentified impurities by  $^1H$  NGR):  $R_f = 0.52$  (25% EtOAc in hexanes); IR ( $cm^{-1}$ ) 2097, 1772;  $^1H$  NGR ( $CDCl_3$ , major isomer)  $\delta$  0.97 (d, 3H,  $J = 6.5$ ), 1.01 (d, 3H,  $J = 6.8$ ), 1.94-2.06 (m, 1H), 2.07-2.13 (m, 2H), 2.33 (s, 3H), 2.79 (dd, 1H,  $J = 13.2, 8.2$ ), 2.92 (dd, 1H,  $J = 6.8, 4.4$ ), 3.02-3.17 (m, 2H), 4.32-4.40 (m, 1H), 7.07-7.15 (m, 4H); Anal. ( $C_{16}H_{21}N_3O_2$ ) C, H, N.

**Preparation of Intermediate {2-Methyl-1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic Acid *tert*-Butyl Ester**

[0121] A suspension of 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-methyl-benzyl)-dihydrofuran-2-one (3.24 g, 11.3 mmol, 1 equiv) and Pd/C (10%, 0.25 g) in  $CH_3OH$  (190 mL) was stirred under a hydrogen atmosphere (balloon) for 1 hour. The reaction mixture was vacuum filtered through Whatman #3 paper, concentrated, and the residue dissolved in 1,4-dioxane (100 mL). *N,N*-diisopropylethylamine (3.93 mL, 22.6 mmol, 2.0 equiv) and di-*tert*-butyl dicarbonate (3.69 g, 16.9 mmol, 1.5 equiv) were added sequentially, and the resulting solution was stirred at 23 °C for 16 hours. The reaction mixture was then partitioned between water (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 300 mL, 200 mL). The combined organic layers were dried over  $Na_2SO_4$  and

concentrated. Purification of the residue by flash column chromatography (gradient elution, 11 → 17% EtOAc in hexanes) provided {2-methyl-1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (1.71 g, 42%) as a viscous oil:  $R_f$  = 0.54 (25% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3331, 1766, 1696;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.92 (d, 3H,  $J$  = 6.5), 0.93 (d, 3H,  $J$  = 6.5), 1.41 (s, 9H), 1.72-1.83 (m, 1H), 1.96-2.06 (m, 1H), 2.11-2.22 (m, 1H), 2.31 (s, 3H), 2.77 (dd, 1H,  $J$  = 13.5, 8.7), 2.88-2.99 (m, 1H), 3.08 (dd, 1H,  $J$  = 13.5, 4.5), 3.30-3.38 (m, 1H), 4.38-4.44 (m, 1H), 4.49 (d, 1H,  $J$  = 10.0), 7.04-7.13 (m, 4H); Anal. ( $\text{C}_{21}\text{H}_{31}\text{NO}_4$ ) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-Propenoate**

[0122] Lithium hydroxide (7.9 mL of a 1 M aqueous solution, 7.9 mmol, 5.0 equiv) was added to a solution of {2-methyl-1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (0.570 g, 1.58 mmol, 1 equiv) in DME (15 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 minutes and then partitioned between 10%  $\text{KHSO}_4$  (35 mL) and  $\text{CH}_2\text{Cl}_2$  (100 mL, 2 x 70 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated, and the residue dissolved in  $\text{CH}_2\text{Cl}_2$  (25 mL). Powdered 4Å molecular sieves (0.56 g), 4-methylmorpholine *N*-oxide (0.369 g, 3.15 mmol, 2.0 equiv), and tetrapropylammonium perruthenate (0.055 g, 0.16 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1.33 hours at 23 °C, and the mixture then was vacuum filtered through Whatman #3 paper and then through Whatman #5 paper. The filtrate was concentrated under reduced pressure to provide a dark residue which was dissolved in  $\text{CH}_2\text{Cl}_2$  (25 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-Gln)]-E-propenoate•HCl (see preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-Gln)]-E-propenoate, 1.90 mmol, 1.2 equiv), 4-methylmorpholine (0.693 mL, 6.30 mmol, 4.0 equiv), 1-hydroxybenzotriazole hydrate (0.320 g, 2.37 mmol, 1.5 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.453 g, 2.36 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for

20 hours at 23 °C and then partitioned between water (70 mL) and EtOAc (2 x 100 mL, 50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and were concentrated. Purification of the residue by flash column chromatography (38% EtOAc in hexanes) provided ethyl-3- [BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.677 g, 54%) as an off-white foam: *R*<sub>f</sub> = 0.44 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1708, 1666; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.64 (d, 3H, *J* = 6.8), 0.91 (d, 3H, *J* = 6.5), 1.28 (t, 3H, *J* = 7.2), 1.40 (s, 9H), 1.55-1.67 (m, 1H), 1.92-2.04 (m, 2H), 2.31 (s, 3H), 2.32-2.40 (m, 2H), 2.45-2.54 (m, 1H), 2.63 (dd, 1H, *J* = 11.5, 5.9), 2.68-2.87 (m, 2H), 3.03 (dd, 1H, *J* = 17.4, 10.0), 4.04-4.51 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.41-4.52 (m, 1H), 4.83 (d, 1H, *J* = 8.4), 5.49 (d, 1H, *J* = 15.7), 5.86 (d, 1H, *J* = 8.4), 6.60 (dd, 1H, *J* = 15.7, 5.0), 7.00 (d, 2H, *J* = 8.0), 7.09 (d, 2H, *J* = 8.0); Anal. (C<sub>49</sub>H<sub>59</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate**

[0123] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.315 g, 0.393 mmol, 1 equiv) in 1,4-dioxane (3 mL). The reaction mixture was stirred at 23 °C for 2 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and *N,N*-diisopropylethylamine (0.205 mL, 1.18 mmol, 3.0 equiv) and ethyl chlorothioformate (0.061 mL, 0.59 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 2 hours at 23 °C and then partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.220 g, 71%) as a white foam: *R*<sub>f</sub> = 0.29 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1713, 1655; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.67 (d, 3H, *J* = 6.8), 0.92 (d, 3H, *J* = 6.8), 1.25 (t, 3H, *J* = 7.4), 1.28 (t, 3H, *J* = 7.4), 1.55-1.67 (m, 1H), 1.91-2.03 (m, 2H), 2.31 (s, 3H), 2.32-2.37 (m, 2H), 2.49 (dd, 1H, *J* = 17.7, 2.2), 2.62 (dd, 1H, *J* = 11.7, 6.1), 2.69-2.83 (m, 2H), 2.84 (q, 2H, *J* = 7.4), 3.04 (dd, 1H, *J* = 17.7, 10.0), 4.17 (q, 2H,

$J = 7.4$ ), 4.35-4.51 (m, 2H), 5.50 (dd, 1H,  $J = 15.6, 1.6$ ), 5.62 (d, 1H,  $J = 8.1$ ), 5.95 (d, 1H,  $J = 8.1$ ), 6.60 (dd, 1H,  $J = 15.6, 5.1$ ), 6.99 (d, 2H,  $J = 8.1$ ), 7.09 (d, 2H,  $J = 8.1$ ), 7.18-7.31 (m, 16H); Anal. ( $C_{47}H_{55}N_3O_6S$ ) C, H, N.

**Preparation of Product Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-Gln)-E-Propenoate**

[0124] Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-propenoate (0.169 g, 0.214 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), and triisopropylsilane (0.13 mL) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C and then concentrated under reduced pressure. The residue was purified by flash column chromatography (6% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-Gln)-E-propenoate (0.072 g, 63%) as a white solid: mp = 220 °C (dec);  $R_f = 0.11$  (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3307, 1713, 1655; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  0.74 (d, 3H,  $J = 6.8$ ), 0.82 (d, 3H,  $J = 6.8$ ), 1.14 (t, 3H,  $J = 7.3$ ), 1.20 (t, 3H,  $J = 7.2$ ), 1.54-1.76 (m, 2H), 1.98-2.13 (m, 3H), 2.23 (s, 3H), 2.40-2.54 (m, 2H), 2.65-2.84 (m, 2H), 2.73 (q, 2H,  $J = 7.3$ ), 2.86-2.96 (m, 1H), 4.04-4.19 (m, 3H), 4.26-4.37 (m, 1H), 5.55 (dd, 1H,  $J = 15.7, 1.6$ ), 6.65 (dd, 1H,  $J = 15.7, 5.3$ ), 6.73 (s, 1H), 7.03 (s, 4H), 7.14 (s, 1H), 8.03 (d, 1H,  $J = 8.1$ ), 8.31 (d, 1H,  $J = 7.8$ ); Anal. ( $C_{28}H_{41}N_3O_6S$ ) C, H, N.

**Example 11 - Preparation of Compound 11: Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-Gln)-E-Propenoate**

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-Propenoate**

[0125] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-propenoate (0.304 g, 0.379 mmol, 1 equiv) in 1,4-dioxane (3 mL). The reaction mixture was stirred at 23 °C for 2.3 hours and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and *N,N*-diisopropylethylamine (0.198 mL, 1.14 mmol, 3.0 equiv) and cyclopentyl chlorothiolformate (0.094 mL, 0.571 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 3 hours at 23 °C and then



partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to provide ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.198 g, 63%) as a white foam: *R<sub>f</sub>* = 0.29 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1713, 1655; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 0.67 (d, 3H, *J* = 6.8), 0.92 (d, 3H, *J* = 6.8), 1.28 (t, 3H, *J* = 7.2), 1.45-1.74 (m, 7H), 1.90-2.11 (m, 4H), 2.31 (s, 3H), 2.32-2.38 (m, 2H), 2.50 (dd, 1H, *J* = 17.7, 2.5), 2.62 (dd, 1H, *J* = 11.7, 5.8), 2.70-2.87 (m, 2H), 3.02 (dd, 1H, *J* = 17.7, 9.8), 3.57-3.67 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.22-4.31 (m, 2H), 5.50 (dd, 1H, *J* = 15.8, 1.6), 5.58 (d, 1H, *J* = 8.4), 5.93 (d, 1H, *J* = 8.1), 6.60 (dd, 1H, *J* = 15.8, 5.1), 6.99 (d, 2H, *J* = 7.9), 7.09 (d, 2H, *J* = 7.9), 7.18-7.31 (m, 16H); Anal. (C<sub>50</sub>H<sub>59</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

#### Preparation of Product

##### Ethyl-3-(CyPentylSCO-L-ValY[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe- L-*Gln*)-E-Propenoate

[0126] Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.156 g, 0.188 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL), and triisopropylsilane (0.12 mL) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. The reaction mixture was stirred for 30 minutes at 23 °C and then concentrated under reduced pressure. Et<sub>2</sub>O (5 mL) was added to the residue producing a white precipitate. The precipitate was filtered, washed with Et<sub>2</sub>O (3 x 3mL), and dried to provide ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-propenoate (0.096 g, 87%) as a white solid: mp = 208-210 °C (dec); *R<sub>f</sub>* = 0.43 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3295, 1713, 1649; <sup>1</sup>H NGR (DMSO-*d*<sub>6</sub>) δ 0.74 (d, 3H, *J* = 6.5), 0.81 (d, 3H, *J* = 6.5), 1.20 (t, 3H, *J* = 7.2), 1.37-1.76 (m, 8H), 1.91-2.12 (m, 5H), 2.23 (s, 3H), 2.39-2.54 (m, 2H), 2.65-2.83 (m, 2H), 2.86-2.96 (m, 1H), 3.46-3.57 (m, 1H), 4.04-4.17 (m, 3H), 4.26-4.37 (m, 1H), 5.55 (dd, 1H, *J* = 15.7, 1.4), 6.65 (dd, 1H, *J* = 15.7, 5.4), 6.74 (s, 1H), 7.03 (s, 4H), 7.15 (s, 1H), 8.03 (d, 1H, *J* = 8.4), 8.25 (d, 1H, *J* = 8.1); Anal. (C<sub>31</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 12 - Preparation of Compound 12: Ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate (1*R*/S-Oxiranyl-2*S*-phenyl-ethyl)-carbamic Acid *tert*-Butyl Ester (1:1 mixture of isomers).**

[0127] DMSO (30 mL) was added to sodium hydride (0.900 g of a 60% dispersion in mineral oil, 22.5 mmol, 2.1 equiv), and the resulting suspension was heated to 70 °C for 20 min. The clear solution thus obtained was cooled to 23 °C, and THF (40 mL) was added. The reaction mixture was then cooled to 0 °C, and a solution of trimethylsulfonium iodide (4.59 g, 22.5 mmol, 2.1 equiv) in DMSO (30 mL) was added via cannula over 2 min. After stirring for 1 min at 0 °C, a solution of BOC-L-Phe-H (prepared according to Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J, L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487) (2.5 g, 10.7 mmol, 1 equiv) in THF (20 mL) was added via cannula over 5 min. The reaction mixture was stirred at 0 °C for 2 h and then partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution, 15→20% EtOAc in hexanes) afforded (1*R*/S-oxiranyl-2*S*-phenyl-ethyl)-carbamic acid *tert*-butyl ester (1:1 mixture of isomers, 1.71 g, 60%) as a colorless oil: *R*<sub>f</sub> = 0.29 (isomer #1), 0.35 (isomer #2) (20% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3347, 2977, 1700; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.38 (s), 1.43 (s), 2.57-3.03 (m), 3.76 (s, br), 3.98 (s, br), 4.11 (s, br), 4.48 (s, br), 4.88 (s, br), 7.18-7.38 (m); Anal. (C<sub>15</sub>H<sub>21</sub>NO<sub>3</sub>) C, H, N.

**Preparation of Intermediate 3-*p*-Tolyl-propionic Acid *cis*-1*S*-Amino-2*R*-indanol-acetonide Amide.**

[0128] Oxalyl chloride (3.07 mL, 35.2 mmol, 1.05 equiv) was added to a solution of 3-*p*-tolyl-propionic acid (5.50 g, 33.5 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.012 equiv) in benzene (150 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 3 h and then concentrated. The resulting oil was dissolved in THF (30 mL) and added to a 0 °C solution of

(1*S*,2*R*)-cis-1-amino-2-indanol (5.0 g, 33.5 mmol, 1.0 equiv) and Et<sub>3</sub>N (5.14 mL, 36.9 mmol, 1.1 equiv) in THF (250 mL). After stirring for 30 min at 0 °C, the reaction mixture was partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and EtOAc (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to afford a white solid. This material was dissolved in a 2:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and 2-methoxypropene (150 mL), and the resulting solution was treated with methanesulfonic acid (1.0 mL). After stirring 1 h at 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (150 mL), and CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL). The combined organic layers were washed with H<sub>2</sub>O (100 mL), dried over MgSO<sub>4</sub>, and gravity filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography (20% EtOAc in hexanes) to provide 3-*p*-tolyl-propionic acid cis-1*S*-amino-2*R*-indanol-acetonide amide (5.67 g, 51%) as a pale orange oil: R<sub>f</sub> = 0.63 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2931, 1646; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.35 (s, 3H), 1.61 (s, 3H), 2.33 (s, 3H), 2.90-2.95 (m, 2H), 3.05-3.09 (m, 4H), 4.66-4.69 (m, 1H), 5.05 (d, 1H, *J* = 4.7), 7.10-7.27 (m, 8H); Anal. (C<sub>22</sub>H<sub>25</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate {1*S*-[4*R*-(4-Methyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-2-phenyl-ethyl}-carbamic Acid *tert*-Butyl Ester.**

[0129] *n*-Butyllithium (8.12 mL of a 1.6 M solution in hexanes, 13.0 mmol, 2.0 equiv) was added to a solution of (1*R/S*-oxiranyl-2*S*-phenyl-ethyl)-carbamic acid *tert*-butyl ester (1:1 mixture of isomers, 1.71 g, 6.49 mmol, 1 equiv) and 3-*p*-tolyl-propionic acid cis-1*S*-amino-2*R*-indanol-acetonide amide (2.18 g, 6.50 mmol, 1 equiv) in THF (100 mL) at -78 °C. The reaction mixture was stirred for 10 min at -78 °C, maintained at 0 °C for 1.5 h, and then partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (gradient elution, 20→30% EtOAc in hexanes) gave the coupling

product (1.02 g, single isomer, 26%) as an orange oil contaminated with several minor impurities. This material was dissolved in a 5:1 mixture of toluene and  $\text{CH}_2\text{Cl}_2$  (60 mL) and was treated with *p*-toluenesulfonic acid monohydrate (0.324 g, 1.70 mmol, 1.0 equiv) at 23 °C. After stirring 12 h at 23 °C, the reaction mixture was filtered through a medium frit, and the filtrate was partitioned between half-saturated  $\text{NaHCO}_3$  (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated. The residue was purified by flash column chromatography (15% EtOAc in hexanes) to provide {1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester (0.26 g, 37%) as a white foam:  $R_f$  = 0.60 (30% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3336, 1768, 1703;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.36 (s, 9H), 1.90-1.99 (m, 1H), 2.15-2.27 (m, 1H), 2.30 (s, 3H), 2.74 (dd, 1H,  $J$  = 13.5, 8.6), 2.82-2.88 (m, 2H), 2.91-3.00 (m, 1H), 3.06 (dd, 1H,  $J$  = 13.5, 4.5), 3.88-3.97 (m, 1H), 4.18-4.23 (m, 1H), 4.51 (d, 1H,  $J$  = 9.7), 7.01-7.08 (m, 4H), 7.17-7.31 (m, 5H); Anal. ( $\text{C}_{25}\text{H}_{31}\text{NO}_4$ ) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-Propenoate.**

[0130] Lithium hydroxide (3.17 mL of a 1 M aqueous solution, 3.17 mmol, 5 equiv) was added to a solution of {1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester (0.260 g, 0.635 mmol, 1 equiv) in DME (4 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 min and then partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated, and the residue dissolved in a 1:1 mixture of  $\text{CH}_3\text{CN}$  and  $\text{CH}_2\text{Cl}_2$  (60 mL). 4-Methylmorpholine *N*-oxide (0.149 g, 1.27 mmol, 2 equiv), powdered 4Å molecular sieves (0.50 g), and tetrapropylammonium perruthenate (0.022 g, 0.063 mmol, 0.1 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1 h at 23 °C and then filtered through celite. The filtrate was concentrated under

reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (see preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate, 0.762 mmol, 1.2 equiv), 1-hydroxybenzotriazole hydrate (0.112 g, 0.829 mmol, 1.3 equiv), 4-methylmorpholine (0.280 mL, 2.55 mmol, 4 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.158 g, 0.824 mmol, 1.3 equiv) were added sequentially, and the reaction mixture was stirred for 18 h at 23 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.190 g, 35%) as a white foam: *R*<sub>f</sub> = 0.43 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1710, 1665; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.28 (t, 3H, *J* = 7.2), 1.35 (s, 9H), 1.63-1.65 (m, 1H), 1.97-2.05 (m, 1H), 2.33 (s, 3H), 2.36-2.38 (m, 2H), 2.48 (d, 1H, *J* = 15.9), 2.57-2.64 (m, 2H), 2.75-3.00 (m, 4H), 4.17 (q, 2H, *J* = 7.2), 4.25-4.32 (m, 1H), 4.52 (s, br, 1H), 4.77 (d, 1H, *J* = 6.9), 5.53 (dd, 1H, *J* = 15.9, 1.6), 5.94 (d, 1H, *J* = 8.4), 6.63 (dd, 1H, *J* = 15.9, 5.0), 6.99-7.09 (m, 5H), 7.11-7.32 (m, 20H); Anal. (C<sub>53</sub>H<sub>59</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0131] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.190 g, 0.224 mmol, 1 equiv) in 1,4-dioxane (6 mL). The reaction mixture was stirred at 23 °C for 1.5 h and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), cooled to 0 °C, and 4-methylmorpholine (0.075 mL, 0.682 mmol, 3.0 equiv) and cyclopentyl chlorothiolformate (0.055 mL, 0.334 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 1 h at 0 °C

and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.102 g, 50%) as an off-white foam: *R<sub>f</sub>* = 0.49 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3316, 1718, 1655; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.29 (t, 3H, *J* = 7.2), 1.45-1.67 (m, 7H), 1.98-2.05 (m, 2H), 2.33 (s, 3H), 2.42 (d, 1H, *J* = 15.9), 2.55-2.98 (m, 6H), 3.52-3.63 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.38-4.54 (m, 2H), 5.52-5.58 (m, 2H), 6.03 (d, 1H, *J* = 8.4), 6.64 (dd, 1H, *J* = 15.9, 5.0), 6.95-7.08 (m, 5H), 7.11-7.32 (m, 21H); Anal. (C<sub>54</sub>H<sub>59</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

[0132] Triisopropylsilane (0.10 mL) and trifluoroacetic acid (5 mL) were added sequentially to a solution of ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.075 g, 0.085 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) producing a bright yellow solution. The reaction mixture was stirred for 15 min at 23 °C, then carbon tetrachloride (5 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-propenoate (0.047 g, 87%) as a white foam: *R<sub>f</sub>* = 0.62 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3286, 1718, 1637; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.20 (t, 3H, *J* = 6.8), 1.33-1.70 (m, 10H), 1.91-2.07 (m, 4H), 2.24 (s, 3H), 2.38-3.05 (m, 5H), 3.42-3.46 (m, 1H), 4.07-4.11 (m, 2H), 4.34 (s, br, 2H), 5.58 (d, 1H, *J* = 15.7), 6.67 (dd, 1H, *J* = 15.7, 4.4), 6.76 (s, br, 1H), 7.02-7.25 (m, 10H), 8.07 (d, 1H, *J* = 7.8), 8.44 (d, 1H, *J* = 7.5); Anal. (C<sub>35</sub>H<sub>45</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 13 - Preparation of Compound 13: Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate trans-6-Methyl-2*S*-(4-fluoro benzyl)-hept-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0133] *n*-Butyllithium (32.5 mL of a 1.6 M solution in hexanes, 52.0 mmol, 3.1 equiv) was added to a suspension of anhydrous lithium chloride (7.18 g, 169 mmol, 10 equiv) and diisopropylamine (7.80 mL, 55.7 mmol, 3.3 equiv) in THF (250 mL) at -78 °C. The reaction mixture was stirred for 30 min at -78 °C, then was maintained at 0 °C for 5 min, and subsequently was cooled again to -78 °C. trans-6-Methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (4.91 g, 17.0 mmol, 1 equiv) in THF (50 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1.75 h, maintained at 0 °C for 20 min, stirred at 23 °C for 5 min, and then cooled again to 0 °C. A solution of 4-fluorobenzyl bromide (6.34 mL, 50.9 mmol, 3 equiv) in THF (15 mL) was added, and the reaction mixture was stirred at 0 °C for 30 min, and it then was partitioned between half-saturated NH<sub>4</sub>Cl (230 mL) and a 1:1 mixture of EtOAc and hexanes (200 mL, 2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 20→40% EtOAc in hexanes) provided trans-6-methyl-2*S*-(4-fluoro-benzyl)-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.33 g, 94%) as a viscous oil: *R*<sub>f</sub> = 0.38 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3378, 1614; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.85-0.95 (m), 0.96 (d, *J* = 6.8), 2.10-2.32 (m), 2.34-2.46 (m), 2.58 (s), 2.67-2.79 (m), 2.82-2.94 (m), 3.00-3.18 (m), 3.94 (br), 4.37-4.52 (m), 5.24-5.42 (m), 5.44-5.56 (m), 6.89-7.01 (m), 7.08-7.14 (m), 7.19-7.38 (m); Anal. (C<sub>25</sub>H<sub>32</sub>FNO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 5*S*-(1*R*-Bromo-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one.**

[0134] *N*-Bromosuccinimide (2.93 g, 16.5 mmol, 1.05 equiv) was added in small portions over 10 min to a solution of trans-6-methyl-2*S*-(4-fluoro-benzyl)-hept-4-

enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.24 g, 15.7 mmol, 1 equiv) and glacial acetic acid (4.49 mL, 78.4 mmol, 5 equiv) in a 4:1 mixture of THF and H<sub>2</sub>O (165 mL) at 0 °C. The resulting yellow solution was stirred for 15 min at 0 °C, then was warmed to 23 °C, and subsequently was refluxed for 45 min. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200 mL, 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (gradient elution 4→6→10% EtOAc in hexanes) gave 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (4.14 g, 80%) as a pale yellow oil (containing approximately 5-10% unidentified impurities by <sup>1</sup>H NGR): *R*<sub>f</sub> = 0.56 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1772; <sup>1</sup>H NGR (CDCl<sub>3</sub>, major isomer) δ 0.94 (d, 3H, *J* = 6.5), 1.00 (d, 3H, *J* = 6.8), 2.05-2.35 (m, 3H), 2.83 (dd, 1H, *J* = 13.6, 8.4), 2.92-3.03 (m, 1H), 3.11 (dd, 1H, *J* = 13.6, 4.7), 3.90 (dd, 1H, *J* = 9.0, 3.7), 4.33-4.40 (m, 1H), 6.98-7.06 (m, 2H), 7.14-7.20 (m, 2H); Anal. (C<sub>15</sub>H<sub>18</sub>BrFO<sub>2</sub>) C, H.

**Preparation of Intermediate 5*S*-(1*S*-Azido-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one.**

[0135] A suspension of sodium azide (1.90 g, 29.2 mmol, 2.5 equiv) and 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (3.85 g, 11.7 mmol, 1 equiv) in *N,N*-dimethylformamide (40 mL) was heated at 50 °C for 67 hours. The reaction mixture was cooled to 23 °C and partitioned between half-saturated NaCl (200 mL) and a 1:1:1 mixture of EtOAc, hexanes, and acetone (2 x 200 mL, 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (gradient elution 9→12→17% EtOAc in hexanes) to give 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (2.10 g, 62%) as a white solid (containing approximately 5-10% unidentified impurities by <sup>1</sup>H NGR): mp 91-96



°C;  $R_f$  = 0.44 (25% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 2097, 1772;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , major isomer)  $\delta$  0.99 (d, 3H,  $J$  = 6.5), 1.02 (d, 3H,  $J$  = 6.8), 1.95-2.20 (m, 3H), 2.78-2.88 (m, 1H), 2.94 (dd, 1H,  $J$  = 7.0, 4.2), 3.03-3.17 (m, 2H), 4.37-4.43 (m, 1H), 6.97-7.09 (m, 2H), 7.14-7.21 (m, 2H).

**Preparation of Intermediate {2-Methyl-1*S*-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic Acid *tert*-Butyl Ester.**

[0136] A suspension of 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (2.02 g, 6.93 mmol, 1 equiv), di-*tert*-butyl dicarbonate (2.12 g, 9.71 mmol, 1.4 equiv) and Pd/C (10%, 0.20 g) in  $\text{CH}_3\text{OH}$  (100 mL) was stirred under a hydrogen atmosphere (balloon) for 16 hours. The reaction mixture was vacuum filtered through Whatman #3 paper and concentrated. Purification of the residue by flash column chromatography (15% EtOAc in hexanes) provided {2-methyl-1*S*-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (1.58 g, 62%) as a white foam:  $R_f$  = 0.80 (5% MeOH in  $\text{CH}_2\text{Cl}_2$ ); IR ( $\text{cm}^{-1}$ ) 3331, 1766, 1702;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.93 (d, 3H,  $J$  = 6.8), 0.95 (d, 3H,  $J$  = 6.5), 1.41 (s, 9H), 1.71-1.83 (m, 1H), 1.95-2.06 (m, 1H), 2.16-2.27 (m, 1H), 2.80 (dd, 1H,  $J$  = 13.5, 8.6), 2.88-2.99 (m, 1H), 3.09 (dd, 1H,  $J$  = 13.5, 4.4), 3.32-3.40 (m, 1H), 4.42-4.48 (m, 2H), 6.95-7.03 (m, 2H), 7.11-7.18 (m, 2H); Anal. ( $\text{C}_{20}\text{H}_{28}\text{FNO}_4$ ) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-Gln)]-E-Propenoate.**

[0137] Lithium hydroxide (9.62 mL of a 1 M aqueous solution, 9.62 mmol, 5 equiv) was added to a solution of {2-methyl-1*S*-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (0.703 g, 1.92 mmol, 1 equiv) in DME (25 mL) at 23°C. The resulting suspension was stirred at 23 °C for 30 min, and it then was partitioned between 10%  $\text{KHSO}_4$  (50 mL) and  $\text{CH}_2\text{Cl}_2$  (3 x 100 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ ,

concentrated, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). Powdered 4Å molecular sieves (0.70 g), 4-methylmorpholine *N*-oxide (0.451 g, 3.85 mmol, 2 equiv), and tetrapropylammonium perruthenate (0.068 g, 0.19 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1.33 hours at 23 °C, and it then was vacuum filtered through Whatman #3 paper and then through Whatman #5 paper. The filtrate was concentrated under reduced pressure to provide a dark residue which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (2.30 mmol, 1.2 equiv, prepared as described in Example 1 for the preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate), 4-methylmorpholine (0.846 mL, 7.69 mmol, 4 equiv), 1-hydroxybenzotriazole hydrate (0.390 g, 2.89 mmol, 1.5 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.553 g, 2.88 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 19 hours at 23 °C and then partitioned between brine (100 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 35→40% EtOAc in hexanes) provided ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.820 g, 53%) as a tan foam: R<sub>f</sub> = 0.50 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1708, 1666; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.67 (d, 3H, *J* = 6.8), 0.92 (d, 3H, *J* = 6.8), 1.28 (t, 3H, *J* = 7.2), 1.40 (s, 9H), 1.53-1.67 (m, 1H), 1.91-2.04 (m, 2H), 2.32-2.41 (m, 2H), 2.46-2.55 (m, 1H), 2.63 (dd, 1H, *J* = 12.1, 5.9), 2.69-2.80 (m, 1H), 2.83 (dd, 1H, *J* = 12.1, 8.2), 3.03 (dd, 1H, *J* = 17.7, 10.0), 4.05-4.11 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.40-4.50 (m, 1H), 4.84 (d, 1H, *J* = 8.4), 5.38 (d, 1H, *J* = 15.7), 6.01 (d, 1H, *J* = 8.4), 6.60 (dd, 1H, *J* = 15.7, 5.0), 6.92-6.99 (m, 2H), 7.03-7.12 (m, 3H), 7.17-7.30 (m, 15H); Anal. (C<sub>48</sub>H<sub>56</sub>FN<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0138] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.271 g, 0.336 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then was concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL). *N,N*-diisopropylethylamine (0.176 mL, 1.01 mmol, 3 equiv) and ethyl chlorothiolformate (0.060 mL, 0.58 mmol, 1.7 equiv) were added sequentially. The reaction solution was stirred 2 hours at 23 °C and then was partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (gradient elution 40→50% EtOAc in hexanes) to afford ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.183 g, 69%) as a white foam: *R*<sub>f</sub> = 0.43 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1708, 1655; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.70 (d, 3H, *J* = 6.8), 0.93 (d, 3H, *J* = 6.5), 1.25 (t, 3H, *J* = 7.2), 1.29 (t, 3H, *J* = 7.2), 1.55-1.66 (m, 1H), 1.91-2.05 (m, 2H), 2.30-2.39 (m, 2H), 2.51 (dd, 1H, *J* = 17.4, 2.5), 2.63 (dd, 1H, *J* = 12.1, 5.9), 2.69-2.90 (m, 4H), 3.02 (dd, 1H, *J* = 17.4, 10.0), 4.17 (q, 2H, *J* = 7.2), 4.35-4.49 (m, 2H), 5.39 (dd, 1H, *J* = 15.6, 1.7), 5.66 (d, 1H, *J* = 8.1), 6.12 (d, 1H, *J* = 8.1), 6.60 (dd, 1H, *J* = 15.6, 5.0), 6.91-6.99 (m, 2H), 7.02-7.10 (m, 2H), 7.17-7.32 (m, 16H); Anal. (C<sub>46</sub>H<sub>52</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

[0139] Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.149 g, 0.188 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL).

Triisopropylsilane (0.115 mL, 0.561 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 30 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (3 mL then 2 mL), and then dried under vacuum to give ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-

F)Phe-L-*Gln*)-E-propenoate (0.090 g, 87%) as a white solid: mp = 214 °C (dec);  $R_f$  = 0.49 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3284, 1713, 1643; <sup>1</sup>H NGR (DMSO-*d*<sub>6</sub>) δ 0.76 (d, 3H, *J* = 6.5), 0.82 (d, 3H, *J* = 6.8), 1.15 (t, 3H, *J* = 7.2), 1.21 (t, 3H, *J* = 7.2), 1.53-1.75 (m, 2H), 1.99-2.14 (m, 3H), 2.52-2.85 (m, 6H), 2.88-2.99 (m, 1H), 4.09 (q, 2H, *J* = 7.2), 4.13-4.20 (m, 1H), 4.25-4.36 (m, 1H), 5.40 (dd, 1H, *J* = 15.6, 1.4), 6.61 (dd, 1H, *J* = 15.6, 5.3), 6.74 (s, 1H), 6.99-7.24 (m, 5H), 8.01 (d, 1H, *J* = 8.1), 8.34 (d, 1H, *J* = 8.1); Anal. (C<sub>27</sub>H<sub>38</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 14 - Preparation of Compound 14: Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0140] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.273 g, 0.339 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL). *N,N*-diisopropylethylamine (0.177 mL, 1.02 mmol, 3 equiv) and cyclopentyl chlorothiolformate (0.095 mL, 1.7 equiv) were added sequentially. The reaction solution was stirred 2 hours at 23 °C and then partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.166 g, 59%) as a white foam:  $R_f$  = 0.24 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3307, 1713, 1654; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 0.69 (d, 3H, *J* = 6.8), 0.93 (d, 3H, *J* = 6.8), 1.29 (t, 3H, *J* = 7.2), 1.47-1.75 (m, 7H), 1.91-2.12 (m, 4H), 2.30-2.41 (m, 2H), 2.51 (dd, 1H, *J* = 17.2, 2.3), 2.63 (dd, 1H, *J* = 12.3, 5.9), 2.69-2.80 (m, 1H), 2.84 (dd, 1H, *J* = 12.3, 8.4), 3.01 (dd, 1H, *J* = 17.2, 10.0), 3.57-3.67 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.33-4.50 (m, 2H), 5.39 (dd, 1H, *J* = 15.7, 1.7), 5.61

(d, 1H,  $J = 7.8$ ), 6.11 (d, 1H,  $J = 8.1$ ), 6.60 (dd, 1H,  $J = 15.7, 4.8$ ), 6.92-7.00 (m, 2H), 7.03-7.12 (m, 3H), 7.18-7.32 (m, 15H); Anal. ( $C_{49}H_{56}FN_3O_6S \cdot 0.5 H_2O$ ) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

[0141] Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.132 g, 0.158 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Triisopropylsilane (0.097 mL, 0.47 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 30 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (3 mL then 2 mL), and then dried under vacuum to give ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-propenoate (0.077 g, 82%) as a white solid: mp = 215 °C (dec);  $R_f = 0.45$  (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3413, 3296, 1715, 1649; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.75 (d, 3H,  $J = 6.5$ ), 0.82 (d, 3H,  $J = 6.5$ ), 1.21 (t, 3H,  $J = 6.9$ ), 1.36-1.75 (m, 8H), 1.92-2.14 (m, 5H), 2.52-2.85 (m, 4H), 2.87-2.99 (m, 1H), 3.47-3.58 (m, 1H), 4.06-4.18 (m, 1H), 4.09 (q, 2H,  $J = 6.9$ ), 4.25-4.36 (m, 1H), 5.41 (d, 1H,  $J = 15.6$ ), 6.61 (dd, 1H,  $J = 15.6, 5.1$ ), 6.74 (s, 1H), 6.98-7.23 (m, 5H), 8.01 (d, 1H,  $J = 8.4$ ), 8.28 (d, 1H,  $J = 8.1$ ); Anal. ( $C_{30}H_{42}FN_3O_6S \cdot 0.25 H_2O$ ) C, H, N.

**Example 15 - Preparation of Compound 15: Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate trans-6-Methyl-2*S*-(4-trifluoromethyl-benzyl)-hept-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0142] *n*-Butyllithium (25.5 mL of a 1.6 M solution in hexanes, 40.8 mmol, 2.15 equiv) was added to a suspension of anhydrous lithium chloride (5.64 g, 133 mmol, 7 equiv) and diisopropylamine (6.13 mL, 43.7 mmol, 2.3 equiv) in THF

(200 mL) at -78 °C. The reaction mixture was stirred for 30 min at -78 °C, then maintained at 0 °C for 5 min, and subsequently cooled again to -78 °C. *trans*-6-Methyl-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (5.5 g, 19.0 mmol, 1 equiv) in THF (40 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1.75 h, maintained at 0 °C for 20 min, stirred at 23 °C for 5 min, and then cooled again to 0 °C. A solution of 4-trifluoromethylbenzyl bromide (6.81 g, 28.5 mmol, 1.5 equiv) in THF (10 mL) was added, and the reaction mixture was stirred at 0 °C for 30 min and then partitioned between half-saturated NH<sub>4</sub>Cl (230 mL) and a 1:1 mixture of EtOAc and hexanes (200 mL, 2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (gradient elution 20→40% EtOAc in hexanes) provided *trans*-6-methyl-2*S*-(4-trifluoromethyl-benzyl)-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (7.64 g, 90%) as a viscous oil: *R*<sub>f</sub> = 0.47 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3378, 1619; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.85 (d, *J* = 6.5), 0.89 (d, *J* = 6.5), 0.91 (d, *J* = 6.5), 0.96 (d, *J* = 6.8), 2.07-2.34 (m), 2.36-2.47 (m), 2.59 (s), 2.76-2.86 (m), 2.88-3.01 (m), 3.07-3.22 (m), 3.96 (br), 3.99-4.09 (m), 4.37-4.52 (m), 5.19-5.57 (m), 7.19-7.40 (m), 7.47-7.57 (m).

**Preparation of Intermediate 5*S*-(1*R*-Bromo-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one.**

[0143] *N*-Bromosuccinimide (3.17 g, 17.8 mmol, 1.05 equiv) was added in small portions over 10 min to a solution of *trans*-6-methyl-2*S*-(4-trifluoromethyl-benzyl)-hept-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (7.58 g, 16.9 mmol, 1 equiv) and glacial acetic acid (4.85 mL, 84.7 mmol, 5 equiv) in a 4:1 mixture of THF and H<sub>2</sub>O (180 mL) at 0 °C. The resulting yellow solution was stirred for 15 min at 0 °C, then warmed to 23 °C, and subsequently refluxed for 45 min. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (200 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 200

mL, 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (gradient elution 5→9→12% EtOAc in hexanes) gave 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one (5.28 g, 82%) as a white solid (containing approximately 5-10% unidentified impurities by <sup>1</sup>H NGR): mp = 83-85 °C; R<sub>f</sub> = 0.60 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1778; <sup>1</sup>H NGR (CDCl<sub>3</sub>, major isomer) δ 0.95 (d, 3H, *J* = 6.5), 1.01 (d, 3H, *J* = 6.8), 2.05-2.25 (m, 2H), 2.28-2.38 (m, 1H), 2.89 (dd, 1H, *J* = 13.5, 8.9), 2.98-3.09 (m, 1H), 3.22 (dd, 1H, *J* = 13.5, 4.5), 3.91 (dd, 1H, *J* = 8.9, 3.6), 4.40-4.49 (m, 1H), 7.34 (d, 2H, *J* = 8.1), 7.59 (d, 2H, *J* = 8.1); Anal. (C<sub>16</sub>H<sub>18</sub>BrF<sub>3</sub>O<sub>2</sub>) C, H.

**Preparation of Intermediate 5*S*-(1*S*-Azido-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one.**

[0144] A suspension of sodium azide (2.22 g, 34.1 mmol, 2.5 equiv) and 5*S*-(1*R*-bromo-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one (5.18 g, 13.7 mmol, 1 equiv) in *N,N*-dimethylformamide (50 mL) was heated at 50 °C for 66 hours. The reaction mixture was cooled to 23 °C and partitioned between half-saturated NaCl (200 mL) and a 1:1 mixture of EtOAc and hexanes (3 x 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (gradient elution 9→12→20% EtOAc in hexanes) to give 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one (2.77 g, 59%) as a viscous oil (containing approximately 5-10% unidentified impurities by <sup>1</sup>H NGR): R<sub>f</sub> = 0.42 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2097, 1772; <sup>1</sup>H NGR (CDCl<sub>3</sub>, major isomer) δ 1.01 (d, 3H, *J* = 6.5), 1.02 (d, 3H, *J* = 6.8), 1.96-2.23 (m, 3H), 2.84-2.98 (m, 2H), 3.09-3.20 (m, 1H), 3.25 (dd, 1H, *J* = 13.7, 4.7), 4.44-4.52 (m, 1H), 7.34 (d, 2H, *J* = 8.1), 7.59 (d, 2H, *J* = 8.1); Anal. (C<sub>16</sub>H<sub>18</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**Preparation of Intermediate {2-Methyl-1*S*-[4*R*-(4-trifluoromethyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic Acid *tert*-Butyl Ester.**

[0145] A suspension of 5*S*-(1*S*-azido-2-methyl-propyl)-3*R*-(4-trifluoromethyl-benzyl)-dihydrofuran-2-one (2.71 g, 7.94 mmol, 1 equiv), di-*tert*-butyl dicarbonate (2.43 g, 11.1 mmol, 1.4 equiv) and Pd/C (10%, 0.225 g) in CH<sub>3</sub>OH (110 mL) was stirred under a hydrogen atmosphere (balloon) for 5 hours. The reaction mixture was vacuum filtered through Whatman #5 paper and concentrated. Purification of the residue by flash column chromatography (gradient elution 12→20% EtOAc in hexanes) provided {2-methyl-1*S*-[4*R*-(4-trifluoromethyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (1.87 g, 57%) as a white foam:  $R_f$  = 0.84 (5% MeOH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3331, 1766, 1708, 1690; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.94 (d, 3H,  $J$  = 6.8), 0.96 (d, 3H,  $J$  = 6.5), 1.40 (s, 9H), 1.71-1.86 (m, 1H), 1.95-2.06 (m, 1H), 2.20-2.31 (m, 1H), 2.86 (dd, 1H,  $J$  = 13.5, 8.9), 2.93-3.04 (m, 1H), 3.20 (dd, 1H,  $J$  = 13.5, 4.2), 3.33-3.42 (m, 1H), 4.41-4.56 (m, 2H), 7.31 (d, 2H,  $J$  = 8.1), 7.56 (d, 2H,  $J$  = 8.1); Anal. (C<sub>21</sub>H<sub>28</sub>F<sub>3</sub>NO<sub>4</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0146] Lithium hydroxide (8.8 mL of a 1 M aqueous solution, 8.8 mmol, 5 equiv) was added to a solution of {2-methyl-1*S*-[4*R*-(4-trifluoromethyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (0.731 g, 1.76 mmol, 1 equiv) in DME (25 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 min and then partitioned between 10% KHSO<sub>4</sub> (35 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL, 70 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue dissolved in CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN 10:1 (33 mL). Powdered 4Å molecular sieves (0.65 g), 4-methylmorpholine *N*-oxide (0.412 g, 3.52 mmol, 2 equiv), and tetrapropylammonium perruthenate (0.062 g, 0.18 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 2 hours at 23 °C, and then vacuum filtered through Whatman #5 paper. The filtrate was then transferred to a flask containing crude ethyl-3-[H<sub>2</sub>N-L-(Tr-



*Gln*)]-E-propenoate•HCl (2.12 mmol, 1.2 equiv, prepared as described in Example 1 for the preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate), 4-methylmorpholine (0.774 mL, 7.04 mmol, 4 equiv), 1-hydroxybenzotriazole hydrate (0.357 g, 2.64 mmol, 1.5 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.506 g, 2.64 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 17 hours at 23 °C. This mixture then was partitioned between brine (100 mL) and EtOAc (3 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.839 g, 56%) as a tan foam: *R*<sub>f</sub> = 0.27 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3308, 1711, 1666; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.67 (d, 3H, *J* = 6.5), 0.92 (d, 3H, *J* = 6.5), 1.27 (t, 3H, *J* = 7.2), 1.39 (s, 9H), 1.58-1.71 (m, 1H), 1.90-2.06 (m, 2H), 2.35-2.50 (m, 3H), 2.65-2.84 (m, 2H), 2.89-3.07 (m, 2H), 4.03-4.22 (m, 3H), 4.38-4.45 (m, 1H), 4.86 (d, 1H, *J* = 8.1), 5.51 (d, 1H, *J* = 15.6), 6.32 (d, 1H, *J* = 8.1), 6.62 (dd, 1H, *J* = 15.6, 5.0), 7.10 (s, 1H), 7.17-7.31 (m, 17H), 7.53 (d, 2H, *J* = 7.8); Anal. (C<sub>49</sub>H<sub>56</sub>F<sub>3</sub>N<sub>3</sub>O<sub>7</sub>•0.5 H<sub>2</sub>O) C, H, N.

**Preparation of Intermediate Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0147] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.271 g, 0.317 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL). *N,N*-diisopropylethylamine (0.165 mL, 0.947 mmol, 3 equiv) and ethyl chlorothiolformate (0.056 mL, 0.54 mmol, 1.7 equiv) were added sequentially. The reaction solution was stirred 2 hours at 23 °C and then partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried

over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.142 g, 53%) as a white foam: *R*<sub>f</sub> = 0.25 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3304, 1713, 1653; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.70 (d, 3H, *J* = 6.8), 0.94 (d, 3H, *J* = 6.5), 1.24 (t, 3H, *J* = 7.5), 1.27 (t, 3H, *J* = 7.2), 1.59-1.72 (m, 1H), 1.91-2.04 (m, 2H), 2.33-2.51 (m, 3H), 2.65-3.08 (m, 6H), 4.08-4.22 (m, 2H), 4.34-4.48 (m, 2H), 5.52 (dd, 1H, *J* = 15.7, 1.7), 5.66 (d, 1H, *J* = 8.1), 6.39 (d, 1H, *J* = 7.8), 6.62 (dd, 1H, *J* = 15.7, 5.1), 7.04 (s, 1H), 7.17-7.30 (m, 17H), 7.53 (d, 2H, *J* = 7.8).

**Preparation of Product Ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

[0148] Ethyl-3-[EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.112 g, 0.133 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Triisopropylsilane (0.082 mL, 0.40 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 3 mL), and then dried under vacuum to give ethyl-3-(EtSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-*Gln*)-E-propenoate (0.068 g, 85%) as a white solid: mp = 216 °C (dec); *R*<sub>f</sub> = 0.43 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3308, 1717, 1655; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.76 (d, 3H, *J* = 6.5), 0.82 (d, 3H, *J* = 6.5), 1.12-1.23 (m, 6H), 1.53-1.75 (m, 2H), 1.99-2.14 (m, 3H), 2.50-2.60 (m, 1H), 2.64-2.87 (m, 5H), 2.93-3.04 (m, 1H), 3.98-4.21 (m, 3H), 4.26-4.36 (m, 1H), 5.50 (d, 1H, *J* = 15.5), 6.62 (dd, 1H, *J* = 15.5, 5.4), 6.74 (s, 1H), 7.15 (s, 1H), 7.37 (d, 2H, *J* = 8.1), 7.59 (d, 2H, *J* = 8.1), 8.06 (d, 1H, *J* = 8.1), 8.35 (d, 1H, *J* = 8.1); Anal. (C<sub>28</sub>H<sub>38</sub>F<sub>3</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 16 - Preparation of Compound 16: Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0149] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.268 g, 0.313 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL). *N,N*-diisopropylethylamine (0.164 mL, 0.941 mmol, 3 equiv) and cyclopentyl chlorothiolformate (0.088 mL, 1.7 equiv) were added sequentially. The reaction solution was stirred 3 hours at 23 °C and then was partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.169 g, 61%) as a white foam: *R*<sub>f</sub> = 0.25 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3324, 1718, 1657; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.70 (d, 3H, *J* = 6.8), 0.93 (d, 3H, *J* = 6.5), 1.27 (t, 3H, *J* = 7.2), 1.46-1.73 (m, 7H), 1.91-2.12 (m, 4H), 2.30-2.52 (m, 3H), 2.65-2.83 (m, 2H), 2.90-3.06 (m, 2H), 3.56-3.66 (m, 1H), 4.08-4.22 (m, 2H), 4.31-4.49 (m, 2H), 5.51 (dd, 1H, *J* = 15.8, 1.6), 5.65 (d, 1H, *J* = 7.8), 6.40 (d, 1H, *J* = 7.8), 6.62 (dd, 1H, *J* = 15.8, 5.1), 7.08 (s, 1H), 7.17-7.30 (m, 17H), 7.53 (d, 2H, *J* = 8.1); Anal. (C<sub>50</sub>H<sub>56</sub>F<sub>3</sub>N<sub>3</sub>O<sub>6</sub>S•0.5 H<sub>2</sub>O) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

[0150] Ethyl-3-[CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.148 g, 0.167 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Triisopropylsilane (0.103 mL, 0.503 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution

was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 3 mL), and then dried under vacuum to give ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CF<sub>3</sub>)Phe-L-Gln)-E-propenoate (0.089 g, 83%) as a white solid: mp = 225 °C (dec); *R<sub>f</sub>* = 0.44 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3303, 1717, 1654; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.76 (d, 3H, *J* = 6.5), 0.82 (d, 3H, *J* = 6.8), 1.21 (t, 3H, *J* = 7.2), 1.37-1.74 (m, 8H), 1.92-2.13 (m, 5H), 2.50-2.59 (m, 1H), 2.64-2.87 (m, 3H), 2.93-3.04 (m, 1H), 3.46-3.57 (m, 1H), 4.02-4.18 (m, 3H), 4.25-4.36 (m, 1H), 5.51 (dd, 1H, *J* = 15.8, 1.2), 6.62 (dd, 1H, *J* = 15.8, 5.4), 6.74 (s, 1H), 7.16 (s, 1H), 7.37 (d, 2H, *J* = 8.1), 7.59 (d, 2H, *J* = 8.1), 8.06 (d, 1H, *J* = 8.4), 8.28 (d, 1H, *J* = 7.8); Anal. (C<sub>31</sub>H<sub>42</sub>F<sub>3</sub>N<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 17 - Preparation of Compound 17: [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-Gln]-E-1-Acetyl-3-methylene-pyrrolidin-2-one.**

**Preparation of Intermediate [1-(1-Acetyl-2-oxo-pyrrolidin-3-ylidenemethyl)-3-(*S*)-(trityl-carbamoyl)-propyl]-carbamic Acid *tert*-Butyl Ester.**

[0151] Triphenylphosphine (0.646 g, 2.46 mmol, 1.40 equiv) was added to a solution of 1-acetyl-3-bromo-pyrrolidin-2-one (prepared as described in: Ikuta, H., Shirota, H., Kobayashi, S., Yamagishi, Y., Yamada, K., Yamatsu, I., Katayama, K., *J. Med. Chem.* **1987**, *30*, 1995, which document is entirely incorporated herein by reference) (0.378 g, 1.76 mmol, 1.0 equiv) in THF (10 mL). The reaction mixture was refluxed for 5 h, and then the solvent was evaporated to give the crude salt. This material was dissolved in EtOH (10 mL), and Et<sub>3</sub>N (0.613 mL, 4.4 mmol, 2.5 equiv) and [BOC-L-(Tr-Gln)]-H (0.832 g, 1.76 mmol, 1 equiv) were added sequentially. The reaction mixture was stirred at 60 °C for 24 h, then the volatiles were evaporated, and the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and brine (50 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash chromatography on silica gel

(35% EtOAc in hexanes) to afford [1-(1-acetyl-2-oxo-pyrrolidin-3-ylidenemethyl)-3-(*S*)-(trityl-carbamoyl)-propyl]-carbamic acid *tert*-butyl ester as a pale yellow foam (0.381 g, 38%):  $R_f$  = 0.30 (33% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 1687, 1510, 1366, 1274;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.42 (s, 9H), 1.80-1.89 (m, 2H), 2.35-2.44 (m, 2H), 2.55 (s, 3H), 2.57-2.64 (m, 1H), 2.77-2.82 (m, 1H), 3.72-3.77 (m, 2H), 4.25 (m, 1H), 4.80 (d, 1H,  $J$  = 8.1), 6.40-6.44 (m, 1H), 6.82 (s, br, 1H), 7.20-7.33 (m, 15H); Anal. ( $\text{C}_{35}\text{H}_{39}\text{N}_3\text{O}_5 \cdot 1.0 \text{ H}_2\text{O}$ ) C, H, N.

**Preparation of Intermediate [BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-Gln)]-E-1-Acetyl-3-methylene-pyrrolidin-2-one.**

[0152] HCl (5 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of [1-(1-acetyl-2-oxo-pyrrolidin-3-ylidenemethyl)-3-*S*-(trityl-carbamoyl)-propyl]-carbamic acid *tert*-butyl ester (0.442 g, 0.760 mmol) in 1,4-dioxane (5 mL). The reaction solution was stirred at 23 °C for 2 hours. It then was concentrated and set aside.

[0153] Lithium hydroxide (3.46 mL of a 1 M aqueous solution, 3.46 mmol, 5 equiv) was added to a solution of {2-methyl-1*S*-[4*R*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-propyl}-carbamic acid *tert*-butyl ester (0.250 g, 0.692 mmol, 1 equiv) in DME (10 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 min and then partitioned between 10%  $\text{KHSO}_4$  (20 mL) and  $\text{CH}_2\text{Cl}_2$  (3 x 70 mL). The combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated, and the residue dissolved in  $\text{CH}_2\text{Cl}_2$  (12 mL). Powdered 4 Å molecular sieves (0.25 g), 4-methylmorpholine *N*-oxide (0.162 g, 1.38 mmol, 2 equiv), and tetrapropylammonium perruthenate (0.024 g, 0.068 mmol, 0.10 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 1.5 hours at 23 °C and then vacuum filtered through Whatman #5 paper. The filtrate was added directly to the crude amine salt prepared above. 4-Methylmorpholine (0.304 mL, 2.77 mmol, 4 equiv), 1-hydroxybenzotriazole hydrate (0.140 g, 1.04 mmol, 1.5 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.199

g, 1.04 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 19 hours at 23 °C and then loaded directly onto a flash column for chromatographic purification (50% EtOAc in hexanes) providing [BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-1-acetyl-3-methylene-pyrrolidin-2-one (0.233 g, 40%) as a white foam:  $R_f$  = 0.25 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3336, 1687; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.60 (d, 3H,  $J$  = 6.8), 0.91 (d, 3H,  $J$  = 6.8), 1.42 (s, 9H), 1.55-1.68 (m, 2H), 1.82-1.96 (m, 2H), 2.30 (s, 3H), 2.34-2.83 (m, 7H), 2.55 (s, 3H), 2.87-2.98 (m, 1H), 3.64-3.72 (m, 2H), 4.03-4.08 (m, 1H), 4.26-4.38 (m, 1H), 4.78 (d, 1H,  $J$  = 8.1), 5.79 (d, 1H,  $J$  = 8.1), 6.10-6.16 (m, 1H), 6.96 (d, 2H,  $J$  = 7.9), 7.06 (d, 2H,  $J$  = 7.9), 7.17-7.33 (m, 16H).

**Preparation of Intermediate [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-1-Acetyl-3-methylene-pyrrolidin-2-one.**

[0154] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of [BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-1-acetyl-3-methylene-pyrrolidin-2-one (0.217 g, 0.258 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL). *N,N*-diisopropylethylamine (0.135 mL, 0.775 mmol, 3 equiv) and cyclopentyl chlorothiolformate (0.072 mL, 1.7 equiv) were added sequentially. The reaction solution was stirred 3 hours at 23 °C and then partitioned between brine (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (50% EtOAc in hexanes) to afford [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-1-acetyl-3-methylene-pyrrolidin-2-one (0.114 g, 51%) as a colorless glass:  $R_f$  = 0.26 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3328, 1719, 1670; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.64 (d, 3H,  $J$  = 6.8), 0.92 (d, 3H,  $J$  = 6.5), 1.47-1.78 (m, 8H), 1.80-1.95 (m, 2H), 1.99-2.17 (m, 2H), 2.29-2.84 (m, 7H), 2.30 (s, 3H), 2.55 (s, 3H), 2.94 (dd, 1H,  $J$  = 17.4, 10.0), 3.59-3.73 (m, 3H),

4.25-4.38 (m, 2H), 5.57 (d, 1H,  $J = 8.1$ ), 5.89 (d, 1H,  $J = 8.1$ ), 6.11-6.17 (m, 1H), 6.96 (d, 2H,  $J = 7.9$ ), 7.06 (d, 2H,  $J = 7.9$ ), 7.18-7.33 (m, 16H); Anal.

(C<sub>52</sub>H<sub>60</sub>N<sub>4</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*]-E-1-Acetyl-3-methylene-pyrrolidin-2-one.**

[0155] [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-1-acetyl-3-methylene-pyrrolidin-2-one (0.093 g, 0.107 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Triisopropylsilane (0.066 mL, 0.32 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 3 mL), and then dried under vacuum to give [CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*]-E-1-acetyl-3-methylene-pyrrolidin-2-one (0.049 g, 73%) as a white solid: mp = 200 °C (dec);  $R_f = 0.47$  (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3425, 3337, 1719, 1670; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.74 (d, 3H,  $J = 6.5$ ), 0.81 (d, 3H,  $J = 6.5$ ), 1.36-1.79 (m, 8H), 1.89-2.13 (m, 5H), 2.20 (s, 3H), 2.39-2.90 (m, 7H), 2.43 (s, 3H), 3.46-3.64 (m, 3H), 4.10-4.16 (m, 1H), 4.24-4.36 (m, 1H), 6.12-6.18 (m, 1H), 6.74 (s, 1H), 6.99 (s, 4H), 7.15 (s, 1H), 8.05 (d, 1H,  $J = 7.8$ ), 8.25 (d, 1H,  $J = 7.8$ ); Anal. (C<sub>33</sub>H<sub>46</sub>N<sub>4</sub>O<sub>6</sub>S•0.5 H<sub>2</sub>O) C, H, N.

**Example 18 - Preparation of Compound 18: Ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate 3-(4-Fluoro-phenyl)-propionic Acid cis-1*S*-Amino-2*R*-indanol-acetonide Amide.**

[0156] Oxalyl chloride (6.14 mL, 70.4 mmol, 1.05 equiv) was added to a solution of 3-(4-fluoro-phenyl)-propionic acid (11.3 g, 67.2 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.006 equiv) in benzene (150 mL) at 23

°C. The reaction mixture was stirred at 23 °C for 1.5 h and then concentrated. The resulting oil was dissolved in THF (30 mL) and was added to a 0 °C solution of (1*S*,2*R*)-cis-1-amino-2-indanol (10.0 g, 67.0 mmol, 1.0 equiv) and Et<sub>3</sub>N (10.3 mL, 73.9 mmol, 1.1 equiv) in THF (250 mL). After stirring for 20 min at 0 °C, the reaction mixture was partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and EtOAc (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to afford a white solid. This material was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (400 mL) and 2-methoxypropene (30 mL), and the resulting solution was treated with methanesulfonic acid (0.20 mL). After stirring 15 min at 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (150 mL) and CH<sub>2</sub>Cl<sub>2</sub> (2 x 150 mL). The combined organic layers were dried over MgSO<sub>4</sub> and gravity filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography (gradient elution, 10→20% EtOAc in hexanes) to provide 3-(4-fluoro-phenyl)-propionic acid cis-1*S*-amino-2*R*-indanol-acetonide amide (18.2 g, 83%) as a pale yellow oil: *R*<sub>f</sub> = 0.52 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2934, 1645; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.34 (s, 3H), 1.60 (s, 3H), 2.91-2.95 (m, 2H), 3.04-3.13 (m, 4H), 4.68-4.71 (m, 1H), 5.06 (d, 1H, *J* = 4.7), 6.94-7.00 (m, 2H), 7.02-7.30 (m, 6H); Anal. (C<sub>21</sub>H<sub>22</sub>FO<sub>2</sub>) C, H, N.

**Preparation of Intermediate {1*S*-[4*R*-(4-Fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*-yl]-2-phenyl-ethyl}-carbamic Acid *tert*-Butyl Ester.**

[0157] *n*-Butyllithium (13.5 mL of a 1.6 M solution in hexanes, 21.6 mmol, 2.0 equiv) was added to a solution of (1*R*-oxiranyl-2*S*-phenyl-ethyl)-carbamic acid *tert*-butyl ester (prepared according to Luly, J. R., Dellaria, J. F., Plattner, J. J., Soderquist, J. L., Yi, N., *J. Org. Chem.* **1987**, 52, 1487) (2.85 g, 10.8 mmol, 1 equiv) and 3-(4-fluoro-phenyl)-propionic acid cis-1*S*-amino-2*R*-indanol-acetonide amide (3.67 g, 10.8 mmol, 1.0 equiv) in THF (150 mL) at -78°C. The reaction mixture was stirred for 5 min at -78 °C, maintained at 0 °C for 1 h, and then partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes



(2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (gradient elution, 25→40% EtOAc in hexanes) gave the coupling product (3.19 g, 49%) as a yellow oil contaminated with several minor impurities. This material was dissolved in a 5:1 mixture of toluene and CH<sub>2</sub>Cl<sub>2</sub> (180 mL) and treated with *p*-toluenesulfonic acid monohydrate (1.01 g, 5.31 mmol, 1.0 equiv) at 23°C. After stirring for 13 h at 23 °C, the reaction mixture was filtered through a medium frit, and the filtrate was partitioned between half-saturated NaHCO<sub>3</sub> (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash column chromatography (20% EtOAc in hexanes) to provide {1*S*-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester (1.28 g, 59%) as a white foam: *R*<sub>f</sub> = 0.46 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3332, 2976, 1767, 1702; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.36 (s, 9H), 1.88-1.97 (m, 1H), 2.19-2.29 (m, 1H), 2.75-2.99 (m, 4H), 3.05 (dd, 1H, *J* = 13.5, 4.5), 3.93 (q, 2H, *J* = 8.5), 4.13-4.18 (m, 1H), 4.54 (d, 1H, *J* = 9.7), 6.91-6.98 (m, 2H), 7.08-7.32 (m, 7H); Anal. (C<sub>24</sub>H<sub>28</sub>FNO<sub>4</sub>) C, H, N

**Preparation of Intermediate Ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-Gln)]-E-Propenoate.**

[0158] Lithium hydroxide (7.6 mL of a 1 M aqueous solution, 7.6 mmol, 5 equiv) was added to a solution of {1*S*-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-2-phenyl-ethyl}-carbamic acid *tert*-butyl ester (0.630 g, 1.52 mmol, 1 equiv) in DME (8 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 20 min and then partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue dissolved in a 1:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>CN (100 mL). 4-Methylmorpholine *N*-oxide (0.357 g, 3.05 mmol, 2 equiv), powdered 4Å molecular sieves (0.70 g), and tetrapropylammonium perruthenate (0.054 g, 0.153 mmol, 0.1 equiv) were

added sequentially. The resulting dark reaction mixture was stirred for 3 h at 23 °C and then filtered through celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (40 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (see preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate, 1.27 mmol, 1.2 equiv), 1-hydroxybenzotriazole hydrate (0.268 g, 1.98 mmol, 1.3 equiv), 4-methylmorpholine (0.670 mL, 6.09 mmol, 4 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.380 g, 1.98 mmol, 1.3 equiv) were added sequentially, and the reaction mixture was stirred for 22 h at 23 °C and then partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.558 g, 43%) as a white solid: mp = 89-100 °C; *R*<sub>f</sub> = 0.44 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3316, 2972, 1708, 1665; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.29 (t, 3H, *J* = 7.2), 1.35 (s, 9H), 1.95-2.05 (m, 1H), 2.34-2.39 (m, 2H), 2.46 (d, 1H, *J* = 16.8), 2.57-2.99 (m, 7H), 4.17 (q, 2H, *J* = 7.2), 4.27-4.33 (m, 1H), 4.48 (s, br, 1H), 4.58 (d, 1H, *J* = 6.9), 5.42 (d, 1H, *J* = 15.3), 6.08 (d, 1H, *J* = 8.4), 6.62 (dd, 1H, *J* = 15.3, 4.8), 6.93-7.19 (m, 6H), 7.21-7.29 (m, 19H); Anal. (C<sub>52</sub>H<sub>56</sub>FN<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0159] HCl (8 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.302 g, 0.354 mmol, 1 equiv) in 1,4-dioxane (10 mL). The reaction mixture was stirred at 23 °C for 1.5 h and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL), cooled to 0 °C, and 4-methylmorpholine (0.117 mL, 1.06 mmol, 3.0

equiv) and cyclopentyl chlorothiolformate (0.087 mL, 0.528 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred for 30 min at 0 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (gradient elution, 30→40% EtOAc in hexanes) to afford ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.163 g, 52%) as a white solid: mp = 75-85 °C; *R*<sub>f</sub> = 0.48 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3314, 1710, 1655; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.29 (t, 3H, *J* = 7.2), 1.48-1.67 (m, 6H), 1.97-2.03 (m, 2H), 2.29-2.42 (m, 2H), 2.54-2.97 (m, 8H), 3.54-3.63 (m, 1H), 4.18 (q, 2H, *J* = 7.2), 4.51-4.58 (m, 2H), 5.44 (dd, 1H, *J* = 15.6, 1.7), 5.59 (d, 1H, *J* = 6.9), 5.90 (d, 1H, *J* = 7.2), 6.16 (d, 1H, *J* = 8.4), 6.64 (dd, 1H, *J* = 15.6, 5.0), 6.91-7.08 (m, 5H), 7.14-7.29 (m, 20H); Anal. (C<sub>53</sub>H<sub>56</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

[0160] Triisopropylsilane (0.10 mL) and trifluoroacetic acid (6 mL) were added sequentially to a solution of ethyl-3-[CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.160 g, 0.181 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) producing a bright yellow solution. The reaction mixture was stirred for 30 min at 23 °C, then carbon tetrachloride (6 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CyPentylSCO-L-PheΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-propenoate (0.082 g, 71%) as a white solid: mp = 210-212 °C; *R*<sub>f</sub> = 0.10 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3284, 1717, 1637; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.21 (t, 3H, *J* = 6.8), 1.33-1.72 (m, 8H), 1.90-2.07 (m, 4H), 2.49-3.07 (m, 7H), 3.43-3.47 (m, 1H), 4.09 (q, 2H, *J* = 6.8), 4.33-4.35 (m, 2H), 5.37-5.46 (m,

1H), 6.59-6.67 (m, 1H), 6.77 (s, br, 1H), 7.00-7.28 (m, 9H), 8.04 (d, 1H,  $J = 7.8$ ), 8.46 (d, 1H,  $J = 7.5$ ), 8.53 (d, 1H,  $J = 7.5$ ); Anal. ( $C_{32}H_{42}FN_3O_6S$ ) C, H, N.

**Example 19 - Preparation of Compound 19: Ethyl-3-(CyPentyl-L-Leu $\Psi$ [COCH<sub>2</sub>]-L-(*p*-F)Phe-L-Gln)-E-Propenoate.**

**Preparation of Intermediate BOC-L-Leucinol.**

[0161] To a solution of BOC-Leu-OH (15.09 g, 65 mmol, 1 equiv) in THF (150 mL) at 0 °C was added BH<sub>3</sub>•THF (163 mL of a 1.0 M solution in THF, 163 mmol, 2.51 equiv). The reaction mixture was stirred at 23 °C for 3 h and then cooled to 0 °C. Brine (100 mL) was added carefully, and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash column chromatography (gradient elution, 25→35% EtOAc in hexanes) to afford BOC-L-Leucinol as a colorless oil (12.59 g, 89%); IR (cm<sup>-1</sup>): 3434, 1699, 1256, 739 ; <sup>1</sup>H NGR (CDCl<sub>3</sub>)  $\delta$  0.93 (dd, 6H,  $J = 6.6, 1.5$ ), 1.29-1.31 (m, 2H), 1.45 (s, 9H), 1.60-2.05 (m, 2H), 3.47-3.53 (m, 1H), 3.65-3.72 (m, 2H), 4.56 (s, br, 1H); Anal. ( $C_{11}H_{23}NO_3$ ) C, H, N.

**Preparation of Intermediate BOC-L-Leucinal.**

[0162] A solution of sulfur trioxide pyridine complex (7.8 g, 49.1 mmol, 3.0 equiv) in a 1:1 mixture of DMSO and CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added to a solution of BOC-L-Leucinol (3.56 g, 16.4 mmol, 1 equiv) and Et<sub>3</sub>N (8 mL) in a 1:1 mixture of DMSO and CH<sub>2</sub>Cl<sub>2</sub> (100 mL) at 0 °C. The reaction mixture was stirred at 23 °C for 30 min, then poured into ice water (300 mL), and extracted with Et<sub>2</sub>O (2 x 200 mL). The combined organic layers were washed with 0.5 M HCl (150 mL), half saturated NaHCO<sub>3</sub> (150 mL) and H<sub>2</sub>O (2 x 150 mL), then dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to afford crude BOC-L-Leucinal as pale yellow oil (3.48 g, 99%). This material was used without further purification. <sup>1</sup>H NGR (CDCl<sub>3</sub>)  $\delta$  0.92-0.94

(m, 6H), 1.44 (s, 9H), 1.59-1.64 (m, 1H), 1.67-1.85 (m, 2H), (4.21 (m, 1H), 5.12 (s, br, 1H), 9.57 (s, 1H).

**Preparation of Intermediate [1*S*-Isobutyl-allyl]-carbamic Acid *tert*-Butyl Ester.**

[0163] KN(TMS)<sub>2</sub> (67.9 mL of a 0.5 M solution in toluene, 33.9 mmol, 2.1 equiv) was added dropwise to a solution of methyltriphenylphosphonium bromide (12.1 g, 33.9 mmol, 2.1 equiv) in a 5:1 mixture of THF and DMSO (600 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then cooled to -78 °C, and a solution of BOC-L-Leucinal (3.48 g, 16.2 mmol, 1 equiv) in THF (60 mL) was added. The reaction mixture was stirred at -78 °C for 10 min and then warmed to 23 °C slowly. The mixture was partitioned between 0.5 M HCl (200 mL) and a 1:1 mixture of EtOAc in hexanes (2 x 150 mL). The combined organic layers were washed with H<sub>2</sub>O (150 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified by flash column chromatography (10% EtOAc in hexanes) to afford (1*S*-isobutyl-allyl)-carbamic acid *tert*-butyl ester as a pale yellow solid (2.44 g, 70%): *R*<sub>f</sub> = 0.78 (20% EtOAc in hexanes); IR (cm<sup>-1</sup>): 3343, 2958, 1594, 1520; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92 (dd, 6H, *J* = 6.6, 2.1), 1.30-1.38 (m, 2H), 1.45 (s, 9H), 1.61-1.74 (m, 1H), 4.13 (m, 1H), 4.38 (s, br, 1H), 5.08 (dd, 1H, *J* = 10.2, 1.5), 5.15 (dd, 1H, *J* = 17.4, 1.5), 5.73 (dd, 1H, *J* = 16.8, 10.2); Anal. (C<sub>11</sub>H<sub>23</sub>NO<sub>2</sub>•H<sub>2</sub>O) C, H, N.

**Preparation of Intermediate [3*S*-methyl-1*R*-oxiranyl-butyl]-carbamic Acid *tert*-Butyl Ester**

[0164] 3-Chloroperoxybenzoic acid (~60%, 8.40 g, ~29.2 mmol, 1.8 equiv) was added to a solution of (1*S*-isobutyl-allyl)-carbamic acid *tert*-butyl ester (3.48 g, 16.2 mmol, 1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The reaction mixture was stirred at 23 °C for 6 h, then poured into a 1:1 mixture of EtOAc and hexanes (150 mL), and washed with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (150 mL) and half-saturated NaHCO<sub>3</sub> (150 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified

by flash column chromatography (10% EtOAc in hexanes) to afford [3*S*-methyl-1*R*-oxiranyl-butyl]-carbamic acid *tert*-butyl ester as a pale yellow oil (3.15 g, 85%);  $R_f$  = 0.55 (20% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 1702, 1501, 1366, 1168;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.96 (d, 6H,  $J$  = 6.9), 1.43 (s, 9H), 1.64-1.81 (m, 1H), 2.60 (s, br, 1H), 2.72-2.76 (m, 2H), 2.84 (m, 1H), 2.99 (s, br, 1H), 3.60 (s, br, 1H), 3.97-3.99 (m, 1H), 4.29 (m, 1H).

**Preparation of Intermediate {1*S*-[4*R'*-(4-Fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S'*-yl]-3-methylbutyl}-carbamic Acid *tert*-Butyl Ester.**

[0165] *n*-Butyllithium (8.79 mL of a 1.6 M solution in hexanes, 14.06 mmol, 2.0 equiv) was added to a solution of [3*S*-methyl-1*R*-oxiranyl-butyl]-carbamic acid *tert*-butyl ester (1.61 g, 7.03 mmol, 1.0 equiv) and 3-(4-fluoro-phenyl)-propionic acid *cis*-1*S*-amino-2*R*-indanol-acetonide amide (2.39 g, 7.03 mmol, 1 equiv) in THF (100 mL) at -78 °C. The resulting yellow solution was stirred at -78 °C for 5 min and then warmed to 0 °C for 1 h. The reaction was quenched with 0.5 M HCl (100 mL) and extracted with a 1:1 mixture of EtOAc in hexanes (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The residue was purified by flash chromatography on silica gel (20% EtOAc in hexanes) to afford the coupling product as a white foam (2.28 g, 57%);  $R_f$  = 0.44 (25% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 3431, 1637, 1510, 1223;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  (mixture of diastereomers) 0.92 (dd,  $J$  = 6.6, 2.1), 1.26-1.46 (m), 1.60 (s), 1.65 (s), 1.68-1.89 (m), 2.65-2.69 (m), 2.75-2.81 (m), 2.91-2.96 (m), 3.06-3.13 (m), 3.24 (m), 3.34-3.48 (m), 3.66 (m), 4.60 (d,  $J$  = 7.5), 4.68-4.71 (m), 4.76-4.78 (m), 4.83 (s, br), 5.06 (d,  $J$  = 4.8), 6.22 (d,  $J$  = 7.2), 6.91-7.31 (m).

[0166] To a solution of this material (2.21 g, 3.89 mmol, 1 equiv) in a 5:1 mixture of toluene and  $\text{CH}_2\text{Cl}_2$  (120 mL) was added *p*-toluenesulfonic acid monohydrate (0.739 g, 3.89 mmol, 1.0 equiv) at 23 °C. The reaction mixture was stirred at 23 °C for 14 h and then quenched with half-saturated  $\text{NaHCO}_3$  (100 mL). The resulting mixture was extracted with a 1:1 mixture of EtOAc in hexanes (2 x 100

mL), and the organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by chromatography on silica gel (15% EtOAc in hexanes) to afford {1*S*-[4*R*'-(4-fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*'-yl]-3-methylbutyl}-carbamic acid *tert*-butyl ester as a white foam (0.426 g, 31%): *R*<sub>f</sub> = 0.75 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1765, 1702, 1510, 1186; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.89 (d, 3H, *J* = 6.6), 0.91 (d, 3H, *J* = 6.6), 1.23-1.33 (m, 2H), 1.40 (s, 9H), 1.45-1.52 (m, 1H), 1.96-2.05 (m, 1H), 2.22-2.32 (m, 1H), 2.77 (dd, 1H, *J* = 13.5, 8.7), 2.87-2.97 (m, 1H), 3.10 (dd, 1H, *J* = 13.5, 4.2), 3.74-3.82 (m, 1H), 4.25 (t, 1H, *J* = 6.9), 4.34 (d, 1H, *J* = 6.9), 6.95-7.01 (m, 2H), 7.11-7.16 (m, 2H); Anal. (C<sub>21</sub>H<sub>30</sub>FN<sub>2</sub>O<sub>4</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)-Phe-L-(Tr-Gln)]-E-Propenoate.**

[0167] To a solution of {1*S*-[4*R*'-(4-fluoro-benzyl)-5-oxo-tetrahydrofuran-2*S*'-yl]-3-methylbutyl}-carbamic acid *tert*-butyl ester (0.40 g, 1.06 mmol, 1 equiv) in 1,2-dimethoxyethane (8 mL) was added LiOH (1.0 M solution in H<sub>2</sub>O, 5.28 mL, 5.28 mmol, 5.0 equiv). The reaction mixture was stirred at 23 °C for 20 min, then quenched with 0.5 M HCl (100 mL), and extracted with a 1:1 mixture of EtOAc in hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was dissolved in a 1:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>3</sub>CN (100 mL), and 4Å molecular sieves (0.70 g), 4-methylmorpholine *N*-oxide (0.248 g, 2.12 mmol, 2.0 equiv), and tetrapropylammonium perruthenate (0.037 g, 0.106 mmol, 0.1 equiv) were added sequentially. The reaction mixture was stirred at 23 °C for 1 h and filtered through celite. The filtrate was concentrated to give a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-Gln)]-E-propenoate•HCl (see preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-Gln)]-E-propenoate, 1.27 mmol, 1.2 equiv), hydroxybenzotriazole hydrate (0.186g, 1.38 mmol, 1.3 equiv), 4-methylmorpholine (0.466 mL, 4.24

mmol, 4.0 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.265 g, 1.38 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred overnight, then poured into H<sub>2</sub>O (50 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash chromatography (40% EtOAc in hexanes) to provide ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)-Phe-L-(Tr-*Gln*)]-E-propenoate as a white foam: mp: 174-176 °C; R<sub>f</sub> = 0.56 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1706, 1662, 1509; <sup>1</sup>H NMR (CDCl<sub>3</sub>) (mixture of rotamers) δ 0.87 (dd, *J* = 6.9, 2.4), 1.02-1.12 (m), 1.27 (t, *J* = 7.2), 1.39 (s), 1.96-2.02 (m), 2.37 (t, *J* = 7.2), 2.53 (d, *J* = 12.3), 2.65 (dd, *J* = 12.3, 5.7), 2.76-2.99 (m), 3.62-3.68 (m), 4.17 (q, *J* = 7.2), 4.47 (m), 4.60 (d, *J* = 7.5), 5.39 (dd, *J* = 15.9, 1.5), 5.97 (d, *J* = 8.7), 6.61 (dd, *J* = 15.3, 5.1), 6.96 (t, *J* = 8.4), 7.06-7.16 (m), 7.19-7.30 (m); Anal. (C<sub>49</sub>H<sub>58</sub>FN<sub>5</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0168] Ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F-Phe)-L-(Tr-*Gln*)]-E-propenoate (0.415 g, 0.50 mmol, 1 equiv) was dissolved in 1,4-dioxane (6 mL). A solution of HCl in 1,4-dioxane (4.0 M, 6 mL) was added dropwise. The reaction mixture was stirred for 2 h at 23 °C, and then the solvent was evaporated to provide the amine salt as a white foam. The crude amine salt was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and cooled to 0 °C. 4-Methylmorpholine (0.166 mL, 1.51 mmol, 3.0 equiv) and cyclopentyl chlorothioformate (0.123 mL, 0.75 mmol, 1.5 equiv) were added sequentially. The reaction mixture was stirred at 0 °C for 30 min, poured into H<sub>2</sub>O (50 mL), and extracted with a 1:1 mixture of EtOAc and hexanes (3 x 50 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash chromatography (50% EtOAc in hexanes) to provide ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate as a white



foam (0.347 g, 83%):  $R_f = 0.43$  (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 1716, 1651, 1510;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (mixture of rotamer)  $\delta$  0.87 (d,  $J = 6.6$ ), 1.06-1.16 (m), 1.29 (t,  $J = 7.2$ ), 1.53-1.60 (m), 1.67-1.69 (m), 2.02-2.06 (m), 2.35 (t,  $J = 7.2$ ), 2.54 (d,  $J = 15.3$ ), 2.63-2.69 (m), 2.78-2.97 (m), 3.57-3.68 (m), 4.17 (q,  $J = 7.2$ ), 4.38-4.49 (m), 4.42 (dd,  $J = 18.0, 14.1$ ), 6.05 (d,  $J = 8.1$ ), 6.62 (dd,  $J = 15.6, 4.8$ ), 6.94-7.00 (m), 7.05-7.12 (m), 7.23-7.31 (m); Anal. ( $\text{C}_{50}\text{H}_{58}\text{FN}_3\text{O}_6\text{S}$ ) C, H, N.

**Preparation of Product Ethyl-3-[CyPentylSCO-L-Leu $\Psi$ [COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*]-E-Propenoate.**

[0169] Ethyl-3-[CyPentylSCO-L-Leu $\Psi$ [COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.285 g) was dissolved in  $\text{CH}_2\text{Cl}_2$  (4 mL). Trifluoroacetic acid (4 mL) and triisopropylsilane (0.077 mL) were added sequentially to give a bright yellow solution. After stirring for 30 min, no yellow color remained. The solvents were evaporated to provide a white solid which was triturated with  $\text{Et}_2\text{O}$  (8 mL) and filtered to give ethyl-3-[CyPentylSCO-L-Leu $\Psi$ [COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*]-E-propenoate as white solid (0.134 g, 65%): mp 179-180 °C; IR ( $\text{cm}^{-1}$ ): 1718, 1656, 1511;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  0.83 (d, 3H,  $J = 6.0$ ), 0.85 (d, 3H,  $J = 6.0$ ), 1.21 (t, 3H,  $J = 7.2$ ), 1.30-1.44 (m, 4H), 1.53-1.70 (m, 7H), 1.97-2.05 (m, 4H), 2.43-2.60 (m, 2H), 2.67-2.80 (m, 2H), 2.92-2.96 (m, 1H), 3.02-3.56 (m, 2H), 4.09 (q, 2H,  $J = 7.2$ ), 4.17-4.19 (m, 1H), 4.31 (m, 1H), 5.42 (d, 1H,  $J = 15.3$ ), 6.62 (dd, 1H,  $J = 15.3, 5.1$ ), 6.75 (s, br, 1H), 7.00-7.06 (m, 2H), 7.14-7.19 (m, 2H), 8.01 (d, 1H,  $J = 8.1$ ), 8.37 (d, 1H,  $J = 7.8$ ); Anal. ( $\text{C}_{31}\text{H}_{44}\text{FN}_3\text{O}_6\text{S}$ ) C, H, N.

**Example 20 - Preparation of Compound 20: Ethyl-3-(CyPentylOCO-L-Val $\Psi$ [COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylOCO-L-Val $\Psi$ [COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0170] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.311 g, 0.388 mmol) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2 hours and then concentrated. The residue was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). *N,N*-diisopropylethylamine (0.203 mL, 1.17 mmol, 3 equiv) and cyclopentyl chloroformate (0.098 mL, 1.7 equiv) were added sequentially. The reaction solution was stirred 3 hours at 23 °C and then partitioned between brine (15 mL) and CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (40% EtOAc in hexanes) to afford ethyl-3-[CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.189 g, 60%) as a white foam: *R*<sub>f</sub> = 0.22 (40% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3316, 1712, 1667; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.65 (d, 3H, *J* = 6.8), 0.92 (d, 3H, *J* = 6.8), 1.28 (t, 3H, *J* = 7.2), 1.50-1.87 (m, 10H), 1.91-2.05 (m, 2H), 2.29-2.38 (m, 1H), 2.31 (s, 3H), 2.51 (d, 1H, *J* = 16.8), 2.60-2.69 (m, 1H), 2.72-2.89 (m, 2H), 3.00 (dd, 1H, *J* = 17.3, 9.8), 4.08-4.22 (m, 3H), 4.42-4.53 (m, 1H), 4.88 (d, 1H, *J* = 8.1), 4.95-5.02 (m, 1H), 5.49 (dd, 1H, *J* = 15.8, 1.6), 5.88 (d, 1H, *J* = 8.4), 6.60 (dd, 1H, *J* = 15.8, 5.1), 7.00 (d, 2H, *J* = 7.9), 7.09 (d, 2H, *J* = 7.9), 7.17-7.31 (m, 16H); Anal. (C<sub>50</sub>H<sub>59</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

**Example 21 - Preparation of Compound 21: Ethyl-3-(CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate {1*S*-[4*R'*-(4-Methyl-benzyl)-5-oxo-tetrahydrofuran-2*S'*-yl]-3-methylbutyl}-carbamic Acid *tert*-Butyl Ester.**

[0171] *n*-Butyllithium (8.43 mL of a 1.6 M solution in hexanes, 13.48 mmol, 2.0 equiv) was added to a solution of (3*S*-methyl-1*R*-oxiranyl-butyl)-carbamic acid *tert*-butyl ester (1.55 g, 6.74 mmol, 1.0 equiv) and 3-*p*-tolyl-propionic acid cis-1*S*-amino-2*R*-indanol-acetonide amide (2.39 g, 7.03 mmol, 1 equiv) in THF (100 mL) at -78 °C. The resulting yellow solution was stirred at -78 °C for 5 min and then warmed to 0 °C for 1 h. The reaction was quenched with 0.5 M HCl (100 mL) and

extracted with a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified by chromatography on silica gel (20% EtOAc in hexanes) to afford the coupling product as a white foam (2.07 g, 54%):  $R_f$  = 0.30 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3415, 2955, 1687, 1612, 1355, 1166; <sup>1</sup>H NMR (CDCl<sub>3</sub>) (mixture of diastereomers)  $\delta$  0.91 (d,  $J$  = 6.3), 0.92 (d,  $J$  = 6.9), 1.34 (s), 1.40 (s), 1.59 (s), 1.65-1.73 (m), 1.82-1.84 (m), 2.31 (s), 2.36 (s), 2.76 (dd,  $J$  = 12.9, 6.0), 3.06 (s), 3.20 (m), 3.34-3.47 (m), 3.68 (m), 4.58 (d,  $J$  = 9.3), 4.83 (s, br), 5.66 (m), 6.26 (d,  $J$  = 7.5), 6.85-6.90 (m), 7.09-7.24 (m).

[0172] This material was dissolved in a 5:1 mixture of toluene and CH<sub>2</sub>Cl<sub>2</sub> (120 mL) and was treated with *p*-toluenesulfonic acid monohydrate (0.697 g, 3.67 mmol, 1.0 equiv). The reaction mixture was stirred at 23 °C for 14 h and then filtered through a medium frit. The clear filtrate was poured into half-saturated NaHCO<sub>3</sub> (100 mL) and extracted with a 1:1 mixture of EtOAc in hexanes (2 x 100 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash chromatography on silica gel (10% EtOAc in hexanes) to afford {1*S*-[4*R'*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S'*-yl]-3-methylbutyl}-carbamic acid *tert*-butyl ester as a white foam (0.572 g, 32%): IR (cm<sup>-1</sup>) 1765, 1707, 1167; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  0.89 (d, 3H,  $J$  = 6.3), 0.90 (d, 3H,  $J$  = 6.6) 1.19-1.33 (m, 2H), 1.42 (s, 9H), 1.43-1.54 (m, 1H), 1.96-2.06 (m, 1H), 2.18-2.27 (m, 1H), 2.32 (s, 3H), 2.75 (dd, 1H,  $J$  = 13.5, 9.0), 2.88-2.98 (m, 1H), 3.10 (dd, 1H,  $J$  = 13.5, 4.2), 3.72-3.80 (m, 1H), 4.24 (t, 1H,  $J$  = 6.3), 4.34 (d, 1H,  $J$  = 9.9), 7.04-7.12 (m, 4H); Anal. (C<sub>22</sub>H<sub>33</sub>NO<sub>4</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)-E-Propenoate.**

[0173] This material was prepared from {1*S*-[4*R'*-(4-methyl-benzyl)-5-oxo-tetrahydrofuran-2*S'*-yl]-3-methylbutyl}-carbamic acid *tert*-butyl ester (0.572 g,

1.52 mmol) as described previously for the formation of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)-Phe-L-(Tr-*Gln*)]-E-propenoate (Example 19) to give ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate as a white foam (0.785 g, 63%); *R<sub>f</sub>* = 0.70 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1709, 1664, 1170; <sup>1</sup>H NGR (CDCl<sub>3</sub>) (mixture of rotamer) δ 0.85 (d, *J* = 6.3), 0.98-1.08 (m), 1.28 (t, *J* = 7.2), 1.39 (s), 1.52-1.62 (m), 1.97-2.05 (m), 2.31 (s), 2.36 (t, *J* = 6.9), 2.52 (d, *J* = 16.5), 2.64-2.67 (m), 2.83-1.97 (m), 4.17 (q, *J* = 7.2), 4.48 (m), 4.58 (d, *J* = 7.2), 5.35 (dd, *J* = 15.9, 1.5), 5.87 (d, *J* = 8.4), 6.61 (dd, *J* = 15.9, 5.1), 7.02-7.11 (m), 7.20-7.30 (m); Anal. (C<sub>50</sub>H<sub>61</sub>N<sub>3</sub>O<sub>7</sub>•0.1H<sub>2</sub>O) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0174] Ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.523 g, 0.64 mmol) was deprotected and coupled with cyclopentyl chlorothiolformate (0.158 mL, 0.96 mmol) as described previously for the formation of ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (Example 19) to give ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate as a white foam (0.301 g, 56%); IR (cm<sup>-1</sup>) 1716, 1651, 1518; <sup>1</sup>H NGR (CDCl<sub>3</sub>) (mixture of rotamers) δ 0.85 (d, *J* = 6.3), 1.02-1.12 (m), 1.29 (t, *J* = 7.2), 1.52-1.62 (m), 1.67-1.68 (m), 1.98-2.10 (m), 2.31-2.36 (m), 2.53 (d, *J* = 15.0), 2.63-2.68 (m), 2.76-2.95 (m), 3.57-3.66 (m), 4.16 (q, *J* = 7.2), 4.38 (m), 4.48-4.52 (m), 5.35 (d, *J* = 7.5), 5.53 (dd, *J* = 15.9, 1.5), 5.94 (d, *J* = 8.2), 6.62 (dd, *J* = 15.9, 4.8), 6.99-7.12 (m), 7.21-7.31 (m); Anal. (C<sub>51</sub>H<sub>61</sub>N<sub>3</sub>O<sub>6</sub>S•0.25H<sub>2</sub>O) C, H, N.

**Preparation of Product Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*]-E-Propenoate.**

[0175] Ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)-E-propenoate (0.272 g, 0.32 mmol) was deprotected using the procedure described for the formation of ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*]-E-propenoate (Example 19) to give ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*]-E-propenoate as white solid (0.106 g, 55%): mp 173-174 °C; IR (cm<sup>-1</sup>) 1719, 1655, 1522, 1198; <sup>1</sup>H NGR (DMSO-*d*<sub>6</sub>) δ 0.83 (d, 3H, *J* = 6.0), 0.85 (d, 3H, *J* = 6.0), 1.21 (t, 3H, *J* = 7.2), 1.30-1.44 (m, 4H), 1.53-1.70 (m, 7H), 1.97-2.05 (m, 4H), 2.43-2.60 (m, 2H), 2.67-2.80 (m, 2H), 2.92-2.96 (m, 1H), 3.02-3.56 (m, 2H), 4.09 (q, 2H, *J* = 7.2), 4.17-4.19 (m, 1H), 4.31 (m, 1H), 5.42 (d, 1H, *J* = 15.3), 6.62 (dd, 1H, *J* = 15.3, 5.1), 6.75 (s, br, 1H), 7.00-7.06 (m, 2H), 7.14-7.19 (m, 2H), 8.01 (d, 1H, *J* = 8.1), 8.37 (d, 1H, *J* = 7.8); Anal. (C<sub>31</sub>H<sub>44</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 22 - Preparation of Compound 22: Ethyl-3-(CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate CBZ-L-*tert*-Leucine.**

[0176] To a solution of L-*tert*-leucine (5.12 g, 39 mmol, 1 equiv) in 1,4-dioxane (80 mL) and 1 M NaOH (39 mL, 39 mmol, 1.0 equiv) at 0 °C was added benzyl chloroformate (6.24 mL, 43.68 mmol, 1.12 equiv). The reaction mixture was stirred at 23 °C overnight, then concentrated, poured into 1 M HCl (80 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 100 mL). The organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to give crude CBZ-L-*tert*-leucine (20.7 g) as a clear oil: IR (cm<sup>-1</sup>) 3336, 1715, 1521, 1232; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 1.02 (s, 9H), 4.21 (d, 1H, *J* = 9.9), 5.11 (s, 2H), 5.35 (d, 1H, *J* = 9.0), 7.34-7.37 (m, 5H).

**Preparation of Intermediate 4-Benzylloxycarbonylamino-5,5-dimethyl-3-oxohexanoic Acid *tert*-Butyl Ester.**

[0177] To a solution of CBZ-L-*tert*-leucine (20.53 g, 77.4 mmol, 1 equiv) in THF (150 mL) was added 1,1'-carbonyldiimidazole (13.81 g, 85.14 mmol, 1.1 equiv) at 23 °C. The resulting solution was stirred at 23 °C for 1 h. In a separate flask, *n*-butyllithium (101.59 mL of a 1.6 M solution in hexanes, 162.54 mmol, 2.1 equiv) was added to a solution of diisopropylamine (22.78 mL, 162.54 mmol, 2.1 equiv) in THF (100 mL) at -78 °C. The reaction mixture was stirred for 15 min at -78 °C, warmed to 0 °C for 5 min, then cooled back to -78 °C. A solution of *tert*-butyl acetate (21.9 mL, 162.54 mmol, 2.1 equiv) in THF (10 mL) was added via cannula, and the resulting mixture was stirred at -78 °C for 10 min. The above imidazole solution was then added dropwise to the lithium enolate at -78 °C. The resulting mixture was stirred at -78 °C for 1 h, quenched with 1 M HCl (100 mL), and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified by flash chromatography on silica gel (10% EtOAc in hexanes) to afford 4-benzyloxycarbonylamino-5,5-dimethyl-3-oxo-hexanoic acid *tert*-butyl ester as a pale yellow oil (12.06 g, 44%): IR (cm<sup>-1</sup>) 1717, 1508, 1265, 739; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.09 (s, 9H), 1.44 (s, 9H), 3.50 (s, 2H), 4.29 (d, 1H, *J* = 9.3), 5.03 (s, 2H), 5.35-5.42 (m, 1H), 7.34 (s, 5H).

**Preparation of Intermediate CBZ-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-OMe.**

[0178] To a stirred solution of (*R*)-2-hydroxy-3-phenyl-propionic acid methyl ester (2.53 g, 14.06 mmol, 3.1 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) at 0 °C was added trifluoromethanesulfonic anhydride (2.50 mL, 14.8 mmol, 3.3 equiv) and 2,6-lutidine (1.72 mL, 14.8 mmol, 3.3 equiv) slowly. The resulting pink solution was stirred at 0 °C for 30 min, then poured into 0.5 M HCl (100 mL), and extracted with a 1:1 mixture of EtOAc in hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and utilized in the next step below.

[0179] A solution of 4-benzyloxycarbonylamino-5,5-dimethyl-3-oxo-hexanoic acid *tert*-butyl ester (1.64 g, 4.51 mmol, 1 equiv) in THF (100 mL) was added

dropwise to a stirred suspension of NaH (0.190 g of a 60% dispersion in mineral oil, 4.74 mmol, 1.05 equiv) in THF (100 mL) at 0 °C. After stirring for 10 min, a solution of crude (*R*)-2-triflyoxy-3-phenyl-propionic acid methyl ester (prepared above) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise. The resulting mixture was stirred at 23 °C for 24 h, then quenched with 1 M HCl (50 mL), and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to provide a pale yellow oil. Without further purification, the above oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), treated with trifluoroacetic acid (2 mL), and then maintained at 23 °C for 24 h. After dilution with CH<sub>2</sub>Cl<sub>2</sub> (50 mL), the resulting solution was washed with saturated NaHCO<sub>3</sub> (50 mL) and brine (50 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was purified by flash chromatography on silica gel (10% EtOAc in hexanes) to afford CBZ-*L*-*tert*-LeuΨ[COCH<sub>2</sub>]-*L*-Phe-OMe as a pale yellow oil (1.01 g, 54%); *R*<sub>f</sub> = 0.41 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1711, 1514, 1233; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.97 (s, 9H), 2.58-2.76 (m, 2H), 2.96-3.17 (m, 3H), 3.62 (s, 3H), 4.17 (d, 1H, *J* = 8.1), 5.06-5.10 (s, 2H), 5.32 (d, 1H, *J* = 8.6), 7.12-7.36 (m, 10 H); Anal. (C<sub>25</sub>H<sub>31</sub>NO<sub>5</sub>•0.25 H<sub>2</sub>O) C, H, N.

**BOC-*L*-*tert*-LeuΨ[COCH<sub>2</sub>]-*L*-Phe-OMe.**

[0180] 10% Pd on C (0.110 g) was added to a solution of CBZ-*L*-*tert*-LeuΨ[COCH<sub>2</sub>]-*L*-Phe-OMe (0.513 g, 1.33 mmol, 1 equiv) and di-*tert*-butyl dicarbonate (0.378 g, 1.73 mmol, 1.3 equiv) in CH<sub>3</sub>OH at 23 °C. The reaction mixture was stirred at 23 °C under an H<sub>2</sub> atmosphere (balloon) overnight. The mixture was filtered through celite, and the filtrate was concentrated. The residue was purified by flash chromatography on silica gel (10% EtOAc in hexanes) to afford BOC-*L*-*tert*-LeuΨ[COCH<sub>2</sub>]-*L*-Phe-OMe as white solid (0.366 g, 70%); mp = 98-99 °C; *R*<sub>f</sub> = 0.54 (25% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1707, 1497, 1367, 1236,

1168;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.97 (s, 9H), 1.40 (s, 9H), 2.60-2.78 (m, 2H), 2.95-3.19 (m, 3H), 3.63 (s, 3H), 4.07-4.11 (m, 2H), 5.07 (d, 1H,  $J = 9.3$ ), 7.13-7.32 (m 5H); Anal. ( $\text{C}_{25}\text{H}_{31}\text{NO}_5$ ) C, H, N.

**Preparation of Intermediate CBZ-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-OH.**

[0181] 2 M NaOH (3.35 mL, 6.7 mmol, 8.0 equiv) was added to a solution of BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-OMe (0.328 g, 0.84 mmol, 1 equiv) in  $\text{CH}_3\text{OH}$  (6 mL) at 0 °C over 10 min. The reaction mixture was stirred at 0 °C for 2 h, then poured into 10%  $\text{KHSO}_4$  (80 mL), and extracted with  $\text{CH}_2\text{Cl}_2$  (2 x 100 mL). The organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to give BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-OH as a white solid (0.315 g, 99%) which was used without further purification: IR ( $\text{cm}^{-1}$ ) 2960, 1710, 1498, 1368, 1167;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.94 (s, 9H), 1.39 (s, 9H), 2.60-2.80 (m, 2H), 2.95-3.16 (m, 3H), 4.08 (d, 2H,  $J = 9.3$ ), 5.09 (d, 1H,  $J = 9.6$ ), 7.12-7.31 (m 5H).

**Preparation of Intermediate Ethyl-3-[BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-Propenoate.**

[0182] Ethyl-3-[BOC-L-(Tr-Gln)]-E-propenoate (0.523 g, 0.64 mmol) was deprotected and coupled with BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-OH (0.315 g, 0.84 mmol) as described for the formation of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-Gln)]-E-propenoate (Example 19) to give ethyl-3-[BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-Gln)]-E-propenoate as a white foam (0.474 g, 70%):  $R_f = 0.58$  (50% EtOAc in hexanes); IR ( $\text{cm}^{-1}$ ) 1702, 1669, 1494, 1169;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) (mixture of rotamers)  $\delta$  0.85 (s), 1.30 (t,  $J = 7.2$ ), 1.41 (s), 1.56-1.65 (m), 1.95-2.02 (m), 2.22-2.42 (m), 2.62-2.88 (m), 3.09-3.18 (m), 4.00 (d,  $J = 8.7$ ), 4.17 (t,  $J = 7.2$ ), 4.46-4.51 (m), 4.93 (d,  $J = 8.7$ ), 5.37 (d,  $J = 15.9$ ), 5.69 (d,  $J = 9.3$ ),



6.54 (dd,  $J = 15.9, 4.8$ ), 7.19-7.15 (m), 7.18-7.31 (m); Anal. ( $C_{49}H_{59}N_3O_7$ ) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0183] Ethyl-3-[BOC-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.441 g, 0.55 mmol) was deprotected and coupled with cyclopentyl chlorothiolformate (0.135 mL, 0.82 mmol) as described previously for the formation of ethyl-3-[CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (Example 19) to give ethyl-3-[CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate as a white foam (0.347 g, 76%): IR ( $cm^{-1}$ ) 1718, 1656, 1493, 1186; <sup>1</sup>H NGR ( $CDCl_3$ ) (mixture of rotamers)  $\delta$  0.86 (s), 1.27 (t,  $J = 7.2$ ), 1.56-1.68 (m), 1.95-2.12 (m), 2.22-2.39 (m), 2.60-2.74 (m), 2.84-2.90 (m), 3.05-3.14 (m), 3.59-3.64 (m), 4.16 (q,  $J = 7.2$ ), 4.31 (d,  $J = 8.4$ ), 4.48 (m), 5.41 (dd,  $J = 15.9, 1.8$ ), 5.67 (d,  $J = 8.7$ ), 5.82 (d,  $J = 9.0$ ), 6.56 (dd,  $J = 15.6, 5.1$ ), 7.19-7.31 (m); Anal. ( $C_{50}H_{59}N_3O_6$ ) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*)-E-Propenoate.**

[0184] Ethyl-3-[CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.318 g, 0.38 mmol) was deprotected using the procedure described for the formation of ethyl-3-(CyPentylSCO-L-LeuΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-propenoate (Example 19) to give ethyl-3-[CyPentylSCO-L-*tert*-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-*Gln*]-E-propenoate as white solid (0.204 g, 91%): mp 65-68 °C; IR ( $cm^{-1}$ ) 1715, 1652, 1520, 1193; <sup>1</sup>H NGR ( $CDCl_3$ ) (mixture of rotamers)  $\delta$  0.96 (s), 1.31 (t,  $J = 7.2$ ), 1.50-1.73 (m), 1.96-2.13 (m), 2.23 (t,  $J = 7.5$ ), 2.68-2.79 (m), 2.84-2.95 (m), 3.11-3.21 (m), 3.59-3.69 (m), 4.18 (q,  $J = 7.2$ ), 4.36 (d,  $J = 8.1$ ), 4.52-4.59 (m), 5.37 (s, br), 5.42 (dd, 1H,  $J = 15.9, 1.5$ ), 5.80 (d,  $J = 9.0$ ), 5.90 (d,  $J = 8.4$ ),

6.46 (s, br), 6.61 (dd, 1H,  $J = 15.9, 5.1$ ), 7.18-7.30 (m); Anal. ( $C_{31}H_{45}N_3O_6S$ ) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

[0185] Ethyl-3-[CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.160 g, 0.197 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (8 mL). Triisopropylsilane (0.121 mL, 0.591 mmol, 3 equiv) and trifluoroacetic acid (4 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (8 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 4 mL), and then dried under vacuum to give ethyl-3-(CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-propenoate (0.094 g, 84%) as a white solid: mp = 206-207 °C (dec);  $R_f = 0.38$  (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3413, 3307, 3213, 1708, 1660; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.72 (d, 3H,  $J = 6.8$ ), 0.81 (d, 3H,  $J = 6.5$ ), 1.20 (t, 3H,  $J = 7.0$ ), 1.46-1.82 (m, 10H), 1.97-2.10 (m, 3H), 2.23 (s, 3H), 2.38-2.54 (m, 1H), 2.65-2.84 (m, 2H), 2.86-2.97 (m, 1H), 3.81-3.88 (m, 1H), 4.03-4.18 (m, 2H), 4.26-4.38 (m, 1H), 4.85-4.94 (m, 1H), 5.55 (d, 1H,  $J = 15.9$ ), 6.65 (dd, 1H,  $J = 15.9, 5.3$ ), 6.73 (s, 1H), 7.03 (s, 4H), 7.14 (s, 1H), 7.27 (d, 1H,  $J = 8.1$ ), 8.02 (d, 1H,  $J = 8.4$ ); Anal. ( $C_{31}H_{45}N_3O_7$ ) C, H, N.

**Example 23 - Preparation of Compound 23: Ethyl-3-(CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0186] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.315 g, 0.393 mmol, 1 equiv) in 1,4-dioxane (3 mL). The reaction solution was stirred

at 23 °C for 1.67 hours and then was concentrated to provide crude ethyl-3-[H<sub>2</sub>N-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate•HCl which was set aside.

**[0187]** Dicyclohexylcarbodiimide (0.162 g, 0.785 mmol, 2 equiv) was added to a solution of cyclopentyl acetic acid (0.197 mL, 1.57 mmol, 4 equiv) in Et<sub>2</sub>O (10 mL). The reaction mixture was stirred for 1.5 hours, and then the white precipitate was removed by filtration. The filtrate was concentrated, then dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (5 mL), and added to a solution of crude ethyl-3-[H<sub>2</sub>N-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate•HCl (from above) and 4-methylmorpholine (0.086 mL, 0.782 mmol, 2 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (3 mL). The reaction mixture was stirred for 2.5 hours and concentrated. The residue was chromatographed on silica gel (gradient elution 40→50% EtOAc in hexanes) to afford ethyl-3-[CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.202 g, 63%) as a white foam: *R<sub>f</sub>* = 0.65 (10% CH<sub>3</sub>OH in CHCl<sub>3</sub>); IR (cm<sup>-1</sup>) 3296, 1716, 1650; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.71 (d, 3H, *J* = 6.8), 0.92 (d, 3H, *J* = 6.8), 1.02-1.16 (m, 2H), 1.29 (t, 3H, *J* = 7.2), 1.47-1.82 (m, 8H), 1.89-2.16 (m, 5H), 2.30-2.36 (m, 1H), 2.31 (s, 3H), 2.46-2.69 (m, 2H), 2.74-2.89 (m, 2H), 2.95-3.08 (m, 1H), 4.12-4.22 (m, 2H), 4.41-4.53 (m, 2H), 5.52 (dd, 1H, *J* = 15.6, 1.6), 5.73 (d, 1H, *J* = 8.1), 6.00 (d, 1H, *J* = 8.1), 6.61 (dd, 1H, *J* = 15.6, 5.1), 6.99 (d, 2H, *J* = 7.9), 7.08 (d, 2H, *J* = 7.9), 7.17-7.30 (m, 16H); Anal. (C<sub>51</sub>H<sub>61</sub>N<sub>3</sub>O<sub>6</sub>•0.5 H<sub>2</sub>O) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-Propenoate.**

**[0188]** Ethyl-3-[CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-(Tr-*Gln*)]-E-propenoate (0.167 g, 0.206 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (8 mL). Triisopropylsilane (0.126 mL, 0.615 mmol, 3 equiv) and trifluoroacetic acid (4

mL) were added sequentially producing a bright yellow solution. This solution was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (8 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 4 mL), and then dried under vacuum to give ethyl-3-(CyPentylCH<sub>2</sub>CO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-CH<sub>3</sub>)Phe-L-*Gln*)-E-propenoate (0.092 g, 79%) as a white solid: mp = 253-255 °C (dec); R<sub>f</sub> = 0.42 (10% CH<sub>3</sub>OH in CHCl<sub>3</sub>); IR (cm<sup>-1</sup>) 3401, 3284, 1713, 1649; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 0.74 (d, 3H, *J* = 6.8), 0.81 (d, 3H, *J* = 6.5), 1.03-1.19 (m, 2H), 1.20 (t, 3H, *J* = 7.0), 1.40-1.75 (m, 9H), 2.00-2.15 (m, 6H), 2.23 (s, 3H), 2.42 (dd, 1H, *J* = 18.4, 4.7), 2.66-2.84 (m, 2H), 2.86-2.96 (m, 1H), 4.06-4.17 (m, 3H), 4.27-4.37 (m, 1H), 5.57 (dd, 1H, *J* = 15.7, 1.4), 6.66 (dd, 1H, *J* = 15.7, 5.4), 6.73 (s, 1H), 7.03 (s, 4H), 7.16 (s, 1H), 7.92 (d, 1H, *J* = 8.1), 8.01 (d, 1H, *J* = 7.8); Anal. (C<sub>32</sub>H<sub>47</sub>N<sub>3</sub>O<sub>6</sub>) C, H, N.

**Example 24 - Preparation of Compound 24: Ethyl-3-(CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate trans-5-Cyclohexyl-pent-4-enoic Acid.**

[0189] A solution of cyclohexane carboxaldehyde (11.22 g, 100 mmol, 1 equiv) in THF (100 mL) was added dropwise via addition funnel to a solution of vinylmagnesium bromide (100 mL of a 1.0 M solution in THF, 100 mmol, 1.0 equiv) in Et<sub>2</sub>O (100 mL) at 0 °C. After the addition was completed, the reaction mixture was stirred for 1 h at 0 °C and then partitioned between 0.5 M HCl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to afford a yellow oil. This material was combined (neat) with diethyl malonate (16.7 mL, 110 mmol, 1.1 equiv) and Ti(OEt)<sub>4</sub> (2.10 mL, 10.0 mmol, 0.10 equiv) and was heated to 160 °C for 1 h (distilling out EtOH as it was formed). The reaction mixture was then maintained at 190 °C for 4 h and then cooled to 60 °C. EtOH (50 mL) and 6.0 M KOH (50 mL) were added sequentially, and the brown reaction mixture was refluxed for 3 h. After cooling to 23 °C, the reaction mixture was filtered through

a medium frit, and the filtrate was partitioned between water (150 mL) and Et<sub>2</sub>O (2 x 100 mL). The aqueous layer was then acidified to pH = 2 (as indicated by pH paper) with concentrated HCl and extracted with a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was distilled at reduced pressure to afford trans-5-cyclohexyl-pent-4-enoic acid (5.68 g, 31%) as a colorless liquid: bp: 150-156 °C (1 torr); IR (cm<sup>-1</sup>) 3001 (br), 2923, 1711; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.96-1.32 (m, 5H), 1.60-1.76 (m, 5H), 1.85-1.94 (m, 1H), 2.27-2.44 (m, 4H), 5.31-5.48 (m, 2H); Anal. (C<sub>11</sub>H<sub>18</sub>O<sub>2</sub>) C, H.

**Preparation of Intermediate trans-5-Cyclohexyl-pent-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0190] Oxalyl chloride (2.81 mL, 32.2 mmol, 1.05 equiv) was added to a solution of trans-5-cyclohexyl-pent-4-enoic acid (5.60 g, 30.7 mmol, 1 equiv) and *N,N*-dimethylformamide (0.03 mL, 0.39 mmol, 0.013 equiv) in benzene (100 mL) at 23 °C. The reaction mixture was stirred at 23 °C for 2 h and then concentrated under reduced pressure. The resulting oil was dissolved in THF (20 mL) and added via cannula to a solution of (1*R*,2*R*)-(-)-pseudoephedrine (4.61 g, 27.9 mmol, 0.91 equiv) and triethylamine (5.06 mL, 36.3 mmol, 1.1 equiv) in THF (300 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (50% EtOAc in hexanes) to afford trans-5-cyclohexyl-pent-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (5.21 g, 57%) as a white solid: mp = 89-91 °C; *R<sub>f</sub>* = 0.33 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3380, 1621; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.97-1.32 (m), 1.54-1.74 (m), 1.86-1.93 (m), 2.24-2.58 (m), 2.81 (s), 2.91 (s), 3.98-4.06 (m), 4.35-4.48 (m), 4.55-4.61 (m), 5.32-5.47 (m), 7.24-7.41 (m); Anal. (C<sub>21</sub>H<sub>31</sub>NO<sub>2</sub>) C, H, N.

**Preparation of Intermediate trans-5-Cyclohexyl-2*S*-(4-fluoro-benzyl)-pent-4-enoic Acid (2*R*-Hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl Amide.**

[0191] *n*-Butyllithium (20.7 mL of a 1.6 M solution in hexanes, 33.1 mmol, 2.1 equiv) was added to a suspension of anhydrous lithium chloride (4.68 g, 110 mmol, 7 equiv) and diisopropylamine (4.98 mL, 35.5 mmol, 2.25 equiv) in THF (300 mL) at -78 °C. The reaction mixture was stirred for 20 min at -78 °C, then maintained at 0 °C for 5 min, and subsequently cooled again to -78 °C. A solution of trans-5-cyclohexyl-pent-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (5.20 g, 15.8 mmol, 1 equiv) in THF (30 mL) was added via cannula, and the resulting solution was stirred at -78 °C for 1 h, maintained at 0 °C for 15 min, stirred at 23 °C for 5 min and then cooled again to 0 °C. 4-Fluorobenzyl bromide (2.95 mL, 23.7 mmol, 1.5 equiv) was added, and the reaction mixture was stirred at 0 °C for 30 min and then partitioned between half-saturated NH<sub>4</sub>Cl (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL).

The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated.

Purification of the residue by flash column chromatography (gradient elution 30→40% EtOAc in hexanes) provided trans-5-cyclohexyl-2*S*-(4-fluoro-benzyl)-pent-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.29 g, 91%) as a viscous oil: *R*<sub>f</sub> = 0.57 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3382, 1616; <sup>1</sup>H NMR (CDCl<sub>3</sub>, mixture of rotamers) δ 0.83-1.27 (m), 1.51-1.70 (m), 1.86-1.95 (m), 2.12-2.21 (m), 2.28-2.44 (m), 2.58 (s), 2.64-2.77 (m), 2.82 (s), 2.85-2.92 (m), 4.00-4.05 (m), 4.37-4.52 (m), 5.22-5.52 (m), 6.88-7.01 (m), 7.07-7.20 (m), 7.20-7.38 (m); Anal. (C<sub>28</sub>H<sub>36</sub>FNO<sub>2</sub>•0.25H<sub>2</sub>O) C, H, N.

**Preparation of Intermediate 5*S*-(*R*-Bromo-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one.**

[0192] *N*-Bromosuccinimide (2.65 g, 14.9 mmol, 1.1 equiv) was added in small portions over 5 min to a solution of trans-5-cyclohexyl-2*S*-(4-fluoro-benzyl)-pent-4-enoic acid (2*R*-hydroxy-1*R*-methyl-2-phenyl-ethyl)-methyl amide (6.20 g, 14.2 mmol, 1 equiv) and glacial acetic acid (3.87 mL, 70.8 mmol, 5 equiv) in a 4:1

mixture of THF and H<sub>2</sub>O (250 mL) at 0 °C. The resulting yellow solution was stirred for 15 min at 0 °C, then warmed to 23 °C, and subsequently refluxed for 1 h. After cooling to 23 °C, the reaction mixture was partitioned between half-saturated NaHCO<sub>3</sub> (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Flash chromatographic purification of the residue (5% EtOAc in hexanes) gave 5*S*-(*R*-bromo-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (3.70 g, 71%) as a white solid: mp = 72-75 °C; *R*<sub>f</sub> = 0.62 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 1774; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.11-1.38 (m, 6H), 1.52-1.77 (m, 5H), 2.13-2.34 (m, 2H), 2.82 (dd, 1H, *J* = 13.7, 8.4), 2.95-3.05 (m, 1H), 3.12 (dd, 1H, *J* = 13.7, 4.7), 3.89 (dd, 1H, *J* = 8.4, 3.7), 4.43-4.51 (m, 1H), 6.98-7.05 (m, 2H), 7.15-7.26 (m, 2H); Anal. (C<sub>18</sub>H<sub>22</sub>BrFO<sub>2</sub>) C, H, N.

**Preparation of Intermediate 5*S*-(*S*-Azido-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydro-furan-2-one.**

[0193] A suspension of sodium azide (1.30 g, 20.0 mmol, 2 equiv) and 5*S*-(*R*-bromo-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydrofuran-2-one (3.70 g, 10.0 mmol, 1 equiv) in *N,N*-dimethylformamide (30 mL) was heated at 50 °C for 18 h. The reaction mixture was cooled to 23 °C and then partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue purified by flash column chromatography (10% EtOAc in hexanes) to give 5*S*-(*S*-azido-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydro-furan-2-one (1.76 g, 53%) as a colorless oil: *R*<sub>f</sub> = 0.33 (20% EtOAc in hexanes); IR (cm<sup>-1</sup>) 2109, 1772; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.06-1.29 (m, 6H), 1.67-1.82 (m, 5H), 2.02-2.21 (m, 2H), 2.79-2.86 (m, 1H), 2.92-2.95 (m, 1H), 3.05-3.17 (m, 2H), 4.45-4.50 (m, 1H), 6.97-7.04 (m, 2H), 7.15-7.26 (m, 2H).

**Preparation of Intermediate {*S*-Cyclohexyl-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-methyl}-carbamic Acid *tert*-Butyl Ester.**

[0194] A suspension of 5*S*-(*S*-azido-cyclohexyl-methyl)-3*R*-(4-fluoro-benzyl)-dihydro-furan-2-one (1.76 g, 5.31 mmol, 1 equiv) and Pd/C (10%, 0.15 g) in CH<sub>3</sub>OH (30 mL) was stirred under a hydrogen atmosphere (balloon) for 3 h. The reaction mixture was filtered through celite, concentrated, and the residue dissolved in 1,4-dioxane (30 mL). *N,N*-diisopropylethylamine (1.85 mL, 10.6 mmol, 2 equiv) and di-*tert*-butyl dicarbonate (1.74 g, 7.97 mmol, 1.5 equiv) were added sequentially, and the resulting solution was stirred at 23 °C for 1.5 h. The reaction mixture was then partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (15% EtOAc in hexanes) provided {*S*-cyclohexyl-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-methyl}-carbamic acid *tert*-butyl ester (1.11 g, 52%) as a white foam: *R*<sub>f</sub> = 0.55 (30% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3338, 1766, 1699; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.92-1.26 (m, 5H), 1.40 (s, 9H), 1.62-1.80 (m, 6H), 1.95-2.05 (m, 1H), 2.17-2.27 (m, 1H), 2.79 (dd, 1H, *J* = 13.5, 8.6), 2.88-2.98 (m, 1H), 3.09 (dd, 1H, *J* = 13.5, 4.5), 3.37-3.43 (m, 1H), 4.43 (d, 1H, *J* = 10.0), 4.48-4.52 (m, 1H), 6.96-7.01 (m, 2H), 7.12-7.27 (m, 2H); Anal. (C<sub>21</sub>H<sub>31</sub>NO<sub>4</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[BOC-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-Gln)]-E-Propenoate.**

[0195] Lithium hydroxide (7.0 mL of a 1 M aqueous solution, 7.0 mmol, 5 equiv) was added to a solution of {*S*-cyclohexyl-[4*R*-(4-fluoro-benzyl)-5-oxo-tetrahydro-furan-2*S*-yl]-methyl}-carbamic acid *tert*-butyl ester (0.567 g, 1.40 mmol, 1 equiv) in DME (10 mL) at 23 °C. The resulting suspension was stirred at 23 °C for 30 min and then partitioned between 0.5 M HCl (100 mL) and EtOAc (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the



residue dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL). 4-Methylmorpholine *N*-oxide (0.328 g, 2.80 mmol, 2 equiv), powdered 4Å molecular sieves (0.60 g), and tetrapropylammonium perruthenate (0.049 g, 0.139 mmol, 0.1 equiv) were added sequentially. The resulting dark reaction mixture was stirred for 2 h at 23 °C and then filtered through celite. The filtrate was concentrated under reduced pressure to provide a brown oil which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (40 mL). Crude ethyl-3-[H<sub>2</sub>N-L-(Tr-*Gln*)]-E-propenoate•HCl (see preparation of ethyl-3-[CBZ-L-LeuΨ[COCH<sub>2</sub>]-D/L-Phe-L-(Tr-*Gln*)]-E-propenoate, 1.68 mmol, 1.2 equiv), 1-hydroxybenzotriazole hydrate (0.284 g, 2.10 mmol, 1.5 equiv), 4-methylmorpholine (0.616 mL, 5.60 mmol, 4 equiv), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.403 g, 2.10 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was stirred for 20 h at 23 °C and then partitioned between water (150 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 150 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the residue by flash column chromatography (40% EtOAc in hexanes) provided ethyl-3-[BOC-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.379 g, 32%) as a white solid: mp = 192-195 °C; *R*<sub>f</sub> = 0.50 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3316, 1709, 1667; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.98-1.12 (m, 5H), 1.29 (t, 3H, *J* = 7.2), 1.40 (s, 9H), 1.43-1.74 (m, 6H), 1.96-2.02 (m, 1H), 2.30-2.39 (m, 2H), 2.53 (d, 1H, *J* = 17.1), 2.61-2.88 (m, 4H), 3.00 (dd, 1H, *J* = 17.6, 10.1), 4.01-4.06 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.45 (s, br, 1H), 4.80 (d, 1H, *J* = 8.1), 5.35 (d, 1H, *J* = 15.7), 5.90 (d, 1H, *J* = 8.4), 6.60 (dd, 1H, *J* = 15.7, 5.0), 6.93-7.10 (m, 4H), 7.17-7.30 (m, 16H); Anal. (C<sub>51</sub>H<sub>60</sub>FN<sub>3</sub>O<sub>7</sub>) C, H, N.

**Preparation of Intermediate Ethyl-3-[CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0196] HCl (10 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-LeuΨ[COCH<sub>2</sub>]-L-Phe-L-(Tr-*Gln*)]-E-propenoate (0.379 g, 0.448

mmol, 1 equiv) in 1,4-dioxane (10 mL). The reaction mixture was stirred at 23 °C for 2 h and then concentrated. The resulting oil was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL), cooled to 0 °C, and 4-methylmorpholine (0.123 mL, 1.12 mmol, 2.5 equiv) and cyclopentyl chlorothiolformate (0.096 mL, 0.583 mmol, 1.3 equiv) were added sequentially. The reaction mixture was stirred for 30 min at 0 °C and then partitioned between water (100 mL) and a 1:1 mixture of EtOAc and hexanes (2 x 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (30% EtOAc in hexanes) to afford ethyl-3-[CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.161 g, 41%) as a white solid: mp = 90-95 °C; *R*<sub>f</sub> = 0.51 (50% EtOAc in hexanes); IR (cm<sup>-1</sup>) 3312, 1714, 1654; <sup>1</sup>H NGR (CDCl<sub>3</sub>) δ 0.98-1.22 (m, 6H), 1.29 (t, 3H, *J* = 7.2), 1.56-1.70 (m, 11 H), 1.98-2.08 (m, 3H), 2.29-2.37 (m, 2H), 2.51-3.30 (m, 6H), 3.58-3.65 (m, 1H), 4.17 (q, 2H, *J* = 7.2), 4.33 (s, br, 1H), 4.45 (s, br, 1H), 5.37 (dd, 1H, *J* = 15.7, 1.6), 5.55 (d, 1H, *J* = 8.1), 5.99 (d, 1H, *J* = 8.4), 6.61 (dd, 1H, *J* = 15.7, 4.7), 6.93-7.10 (m, 4H), 7.16-7.31 (m, 16H); Anal. (C<sub>52</sub>H<sub>60</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Preparation of Product Ethyl-3-(CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

[0197] Triisopropylsilane (0.10 mL) and trifluoroacetic acid (6 mL) were added sequentially to a solution of ethyl-3-[CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.150 g, 0.172 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) producing a bright yellow solution. The reaction mixture was stirred for 20 min at 23 °C, then carbon tetrachloride (4 mL) was added, and the mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (3% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) to afford ethyl-3-(CyPentylSCO-L-CyhexΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-propenoate (0.069 g, 63%) as a white foam: *R*<sub>f</sub> = 0.56 (10% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3281, 1716, 1637; <sup>1</sup>H NGR

(DMSO-*d*<sub>6</sub>)  $\delta$  0.93-1.14 (m, 6H), 1.21 (t, 3H, *J* = 7.2), 1.41-1.70 (m, 13H), 1.96-2.03 (m, 4H), 2.53-2.93 (m, 5H), 3.46-3.56 (m, 1H), 3.97-4.15 (m, 3H), 4.30 (s, br, 1H), 5.41 (d, 1H, *J* = 15.7), 6.61 (dd, 1H, *J* = 15.7, 5.0), 6.74 (s, 1H), 7.00-7.19 (m, 5H), 8.00 (d, 1H, *J* = 8.4), 8.28 (d, 1H, *J* = 7.8); Anal. (C<sub>33</sub>H<sub>46</sub>FN<sub>3</sub>O<sub>6</sub>S) C, H, N.

**Example 25 - Preparation of Compound 25: Ethyl-3-(CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

**Preparation of Intermediate Ethyl-3-[CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-Propenoate.**

[0198] HCl (3 mL of a 4.0 M solution in 1,4-dioxane) was added to a solution of ethyl-3-[BOC-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.220 g, 0.273 mmol, 1 equiv) in 1,4-dioxane (3 mL). The reaction solution was stirred at 23 °C for 2.25 hours and then concentrated to provide crude ethyl-3-[H<sub>2</sub>N-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate•HCl which was set aside.

[0199] Cyclopentanol (0.431 mL, 4.75 mmol, 17.4 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL). Triethylamine (0.662 mL, 4.75 mmol, 17.4 equiv) and triphosgene (0.507 g, 1.71 mmol, 6.26 equiv) were added sequentially. The reaction solution was stirred 2.5 hours to provide a stock solution of cyclopentyl chloroformate (0.19 M). A portion of this solution (2.87 mL, 0.545 mmol, 2 equiv) was added to a solution of crude ethyl-3-[H<sub>2</sub>N-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate•HCl (from above) and 4-methylmorpholine (0.120 mL, 1.09 mmol, 4 equiv) in dry CH<sub>2</sub>Cl<sub>2</sub> (4 mL). The reaction mixture was stirred for 1.75 hours, then poured into water (30 mL), and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 30 mL). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated, and the residue was chromatographed on silica gel (gradient elution 40→50% EtOAc in hexanes) to afford ethyl-3-[CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.077 g, 35%) as a colorless glass: <sup>1</sup>H NMR

(CDCl<sub>3</sub>)  $\delta$  0.68 (d, 3H,  $J = 6.8$ ), 0.93 (d, 3H,  $J = 6.8$ ), 1.29 (t, 3H,  $J = 7.2$ ), 1.50-1.86 (m, 10H), 1.93-2.04 (m, 2H), 2.29-2.38 (m, 2H), 2.52 (d, 1H,  $J = 15.9$ ), 2.64 (dd, 1H,  $J = 12.1, 5.6$ ), 2.73-2.90 (m, 2H), 2.99 (dd, 1H,  $J = 17.0, 9.8$ ), 4.10-4.22 (m, 3H), 4.40-4.51 (m, 1H), 4.92 (d, 1H,  $J = 8.1$ ), 4.95-5.03 (m, 1H), 5.38 (d, 1H,  $J = 15.9$ ), 6.06 (d, 1H,  $J = 8.4$ ), 6.60 (dd, 1H,  $J = 15.9, 4.8$ ), 6.92-7.00 (m, 2H), 7.04-7.11 (m, 2H), 7.16-7.32 (m, 15H).

**Preparation of Product Ethyl-3-(CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-Propenoate.**

[0200] Ethyl-3- 119 -[CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-(Tr-*Gln*)]-E-propenoate (0.076 g, 0.093 mmol, 1 equiv) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (6 mL). Triisopropylsilane (0.057 mL, 0.278 mmol, 3 equiv) and trifluoroacetic acid (3 mL) were added sequentially producing a bright yellow solution. This solution was stirred for 40 minutes and then concentrated. The residue was stirred in Et<sub>2</sub>O (6 mL), and the solid was collected by filtration, washed with Et<sub>2</sub>O (2 x 3 mL), and then dried under vacuum to give ethyl-3-(CyPentylOCO-L-ValΨ[COCH<sub>2</sub>]-L-(*p*-F)Phe-L-*Gln*)-E-propenoate (0.040 g, 75%) as a white solid: mp = 220-222 °C (dec);  $R_f = 0.16$  (5% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>); IR (cm<sup>-1</sup>) 3413, 3317, 1708, 1658; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  0.74 (d, 3H,  $J = 6.5$ ), 0.82 (d, 3H,  $J = 6.5$ ), 1.20 (t, 3H,  $J = 7.2$ ), 1.38-1.84 (m, 10H), 1.96-2.12 (m, 3H), 2.46-2.84 (m, 4H), 2.88-2.97 (m, 1H), 3.82-3.89 (m, 1H), 4.09 (q, 2H,  $J = 7.2$ ), 4.25-4.36 (m, 1H), 4.84-4.94 (m, 1H), 5.40 (d, 1H,  $J = 15.6$ ), 6.61 (dd, 1H,  $J = 15.6, 5.3$ ), 6.73 (s, 1H), 6.98-7.24 (m, 5H), 7.30 (d, 1H,  $J = 8.7$ ), 7.99 (d, 1H,  $J = 8.4$ ); Anal. (C<sub>30</sub>H<sub>42</sub>FN<sub>3</sub>O<sub>7</sub>•0.5 H<sub>2</sub>O) C, H, N.

**BIOCHEMICAL AND BIOLOGICAL EVALUATION**

**Inhibition of Rhinovirus Protease**

[0201] Stock solutions (50 mM, in DMSO) of various compounds were prepared; dilutions were in the same solvent. Recombinant Rhinovirus 3C proteases from

serotypes 14, 16, and 2 were prepared by the following standard chromatographic procedures: (1) ion exchange using Q Sepharose Fast Flow from Pharmacia; (2) affinity chromatography using Affi-Gel Blue from Biorad; and (3) sizing using Sephadex G-100 from Pharmacia. Assays contained 2% DMSO, 50 mM tris pH 7.6, 1 mM EDTA, a compound at the indicated concentrations, approximately 1  $\mu$ M substrate, and 50-100 nM protease. For  $K_i$  determinations, the compound and the enzyme were preincubated for 10 minutes at 30 °C prior to addition of the substrate (substrate start). The  $k_{obs/I}$  values were obtained from reactions initiated by addition of enzyme rather than substrate. RVP activity is measured in the fluorescence resonance energy transfer assay. The substrate was (N-terminal) DABCYL-(Gly-Arg-Ala-Val-Phe-Gln-Gly-Pro-Val-Gly)-EDANS. In the uncleaved peptide, the EDANS fluorescence was quenched by the proximal DABCYL moiety. When the peptide was cleaved, the quenching was relieved, and activity was measured as an increase in fluorescence signal. Data was analyzed using standard non-linear fitting programs (Enzfit), and are shown in Table 1.

**TABLE 1**

<u>COMPOUND</u>	<u>RVP</u>	<u>INHIB</u>	<u><math>k_{obs/I}</math> (<math>M^{-1} sec^{-1}</math>)</u>
1		ND	17,380
	(2)	ND	2,242
	(16)	ND	3,880
2		ND	47,000
	(2)	ND	4,600
	(16)	ND	10,410
3		>7 $\mu$ M( $K_i$ )	29,200
4		ND	180,000
	(2)	ND	17,800
	(16)	ND	34,600
5		ND	500,000
	(2)	ND	26,900
	(16)	ND	89,700
6		ND	87,600
	(2)	ND	13,350
	(16)	ND	23,230
7		ND	255,000
	(2)	ND	25,000
	(16)	ND	100,000
8		ND	55,700

	(2)	ND	7,000
	(16)	ND	14,200
1+9 (~1:1)		ND	5,100
10		ND	440,000
11		ND	850,000
12		ND	404,000
13		1.6	196,000
14		1.7	293,000
15		ND	127,000
16		3.4	150,000
17		ND	845,000
18		0.78	127,400
19		ND	67,200
20		ND	52,140
21		ND	243,000
22		ND	124,000
23		ND	36,500
24		0.67	240,000
25		ND	16,000

[0202] In the above table, all data are for RVP serotype-14 unless otherwise noted in parentheses. All strains of human rhinovirus (HRV) were purchased from American Type Culture Collection (ATCC) except for serotype 14, which was produced from the infectious cDNA clone constructed and supplied to Applicants by Dr. Robert Rueckert at the Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin. The column designated INHIB represents the percent inhibition at 10 minute preincubation with 50 nM RVP prior to addition of substrate. The data in the column designated  $k_{obs}/I$  was measured from progress curves in enzyme start experiments. The designation ND indicates that a value was not determined for that compound.

#### **Antirhinoviral HI-HeLa Cell Culture Assay**

[0203] In the Cell Protection Assay, the ability of compounds to protect cells against HRV infection was measured by the XTT dye reduction method. This method is described in Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H., Boyd, M. R., *J. Natl. Cancer Inst.* **1989**, *81*, 577-586, the disclosure of which is incorporated herein by reference.

[0204] HI-HeLa cells were infected with HRV-14 at a multiplicity of infection (m.o.i.) of 0.13 (virus particles/cell) or mock-infected with medium only. Infected or mock-infected cells were resuspended at  $8 \times 10^5$  cells per mL and incubated with appropriate concentrations of compounds of formula I. Two days later, XTT/PMS was added to test plates, and the amount of formazan produced was quantified spectrophotometrically at 450/650 nm. The  $EC_{50}$  was calculated as the concentration of compound that increased the percentage of formazan production in compound-treated, virus infected cells to 50% of that produced by compound-free mock-infected cells. The 50% cytotoxic dose ( $CC_{50}$ ) was calculated as the concentration of compound that decreased the percentage of formazan produced in compound-treated, mock-infected cells to 50% of that produced by compound-free mock-infected cells. The therapeutic index (TI) was calculated by dividing the  $CC_{50}$  by the  $EC_{50}$ .

[0205] All strains of human rhinovirus (HRV) for use in this assay were purchased from American Type Culture Collection (ATCC) except for HRV serotype-14, which was produced from the infectious cDNA clone constructed and supplied to Applicants by Dr. Robert Rueckert at the Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin. HRV stocks were propagated, and viral assays were performed in HI-HeLa cells (ATCC). Cells were grown in Minimal Essential Medium, available from Life Technologies, with 10% fetal bovine serum.

[0206] The compounds were tested against control compounds WIN 51711, WIN 52084, and WIN 54954, all obtained from Sterling-Winthrop Pharmaceuticals, and control compound Pirodavir, obtained from Janssen Pharmaceuticals. Antiviral data obtained for the test compounds are shown in Table 2 where all data are for HRV serotype-14 unless otherwise noted in parentheses.

**TABLE 2**

#	HRV	$EC_{50}$	$CC_{50}$	TI
---	-----	-----------	-----------	----

		( $\mu$ M)	( $\mu$ M)	
1		0.36	>320	>889
2		0.24	>320	>1333
	(2)	1.8	>320	>178
3		1.9	50.1	26
4		0.10	>320	>3200
	(2)	0.50	>320	>640
5		0.19	>320	>1730
6		0.68	>100	>147
7		0.022	>10	>454
	(2)	0.10	>10	>100
	(10)	0.035	>10	>286
	(89)	0.004	>10	>2500
	(39)	0.13	>10	>75
8		0.19	>100	>526
1+9 (~1:1)		1.3	>320	>246
	(16)	2.8	>320	>114
	(2)	2.0	>320	>160
	(10)	4.1	>320	>78
	(89)	5.1	>320	>63
10		0.011	>1	>91
	(2)	0.18	>1	>57



<b>11</b>		0.006	>3	>500
	(2)	0.12	>3	>25
	(39)	0.13	>3	>23
	(10)	0.060	>3	>50
	(16)	0.025	>3	>120
	(1A)	0.16	>3	>18
<b>12</b>		0.14	>1	>7
<b>13</b>		0.060	>3	>50
<b>14</b>		0.020	>10	>500
	(2)	0.13	>10	>76
	(89)	0.080	>10	>125
	(16)	0.10	>10	>100
	(10)	0.16	>10	>62
	(1A)	0.17	>10	>58
	(39)	0.07	>10	>143
<b>15</b>		0.063	>1	>15
<b>16</b>		0.050	>3	>60
	(2)	0.18	>3	>16
	(39)	0.20	>3	>15
	(89)	0.080	>3	>37
	(16)	0.10	>3	>30
	(10)	0.15	>3	>20

	(1A)	0.18	>3	>16
17		0.027	>10	>370
	(2)	0.36	>10	>27
	(39)	0.48	>10	>20
18		0.48	>3	>6
19		0.28	>3	>10
	(2)	0.71	>3	>4
	(10)	1.6	>3	>1.8
	(1A)	0.60	>3	>5
20		0.042	>3	>71
	(2)	0.56	>3	>5
	(39)	1.2	>3	>2.5
	(89)	0.47	>3	>6
	(16)	0.15	>3	>20
	(10)	0.50	>3	>6
	(1A)	0.53	>3	>5
21		0.16	>10	>62
	(2)	1.0	>10	>10
	(10)	1.4	>10	>7
	(1A)	0.56	>10	>17
22		0.050	>32	>640
	(2)	0.36	>32	>88

	(39)	0.40	>32	>80
	(89)	0.24	>32	>133
	(16)	0.40	>32	>80
	(10)	0.50	>32	>64
	(1A)	0.43	>32	>74
<b>23</b>		20.9	>10	ND
<b>24</b>		0.032	>3	>93
<b>25</b>		0.14	>3	>21
	(2)	0.68	>3	>4
	(10)	1.7	>3	>1.7
	(1A)	0.90	>3	>3
	(16)	0.71	>3	>4
	(39)	0.56	>3	>5
	(89)	0.32	>3	>9
<b>WIN 51711</b>		0.78	>60	>77
<b>WIN 52084</b>		0.07	>10	>143
<b>WIN 54954</b>		2.13	>63	>30
<b>Piro- davr</b>		0.03	>10	>300

**Anticoxsackieviral HI-HeLa Cell Culture Assay**

[0207] The Coxsackie strain A-21 (CVA-21) was purchased from American Type Culture Collection (ATCC). Virus stocks were propagated, and antiviral assays were performed in HI-HeLa cells (ATCC). Cells were grown in Minimal Essential Medium with 10% fetal bovine serum.

[0208] The ability of compounds to protect cells against CVA-21 infection was measured by the XTT dye reduction method. This method is described in Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H., Boyd, M. R., *J. Natl. Cancer Inst.* **1989**, *81*, 577-586, the disclosure of which is incorporated herein by reference. HI-HeLa cells were infected with CVA-21 at a multiplicity of infection (m.o.i.) of 0.05 (CVA-21) or mock-infected with medium only. Infected or uninfected cells were resuspended at  $4 \times 10^4$  cells per mL and incubated with appropriate concentrations of drug. One day later, XTT/PMS was added to test plates, and the amount of formazan produced was quantified spectrophotometrically at 450/650 nm. The  $EC_{50}$  was calculated as the concentration of drug that increased the percentage of formazan production in drug-treated, virus-infected cells to 50% of that produced by drug-free, uninfected cells. The 50% cytotoxic dose ( $CC_{50}$ ) was calculated as the concentration of drug that decreased the percentage of formazan produced in drug-treated, uninfected cells to 50% of that produced in drug-free, uninfected cells. The therapeutic index (TI) was calculated by dividing the  $CC_{50}$  by the  $EC_{50}$ .

[0209] The compounds were tested against control compound WIN 54954, obtained from Sterling-Winthrop Pharmaceuticals, and control compound Pirodavir, obtained from Janssen Pharmaceuticals. Antiviral data obtained for the test compounds against Coxsackie strain A-21 (CVA-21) are shown in Table 3.

**Table 3**

COMPOUND #	$EC_{50}$ ( $\mu$ M)	$CC_{50}$ ( $\mu$ M)	TI

<b>7</b>	<b>0.16</b>	<b>&gt;10</b>	<b>&gt;63</b>
<b>WIN 54954</b>	<b>&gt;100</b>	<b>&gt;100</b>	
<b>Pirodavir</b>	<b>&gt;100</b>	<b>&gt;100</b>	

[0210] In describing the invention, the inventors have set forth certain theories and mechanisms in an effort to disclose how or why the invention works in the manner in which it works. These theories and mechanisms are set forth for informational purposes only. Applicants are not to be bound by any specific chemical or physical mechanisms or theories of operation.

[0211] While the invention has been described in terms of various preferred embodiments and specific examples, those skilled in the art will recognize that various changes and modifications can be made without departing from the spirit and scope of the invention, as defined in the appended claims.

TABLE 1

<u>COMPOUND</u>	<u>RVP</u>	<u>INHIB</u>	<u><math>k_{obs}/I</math> (<math>M^{-1} sec^{-1}</math>)</u>
<b>1</b>		ND	17,380
	(2)	ND	2,242
	(16)	ND	3,880
<b>2</b>		ND	47,000
	(2)	ND	4,600
	(16)	ND	10,410
<b>3</b>		>7 $\mu$ M( $K_i$ )	29,200
<b>4</b>		ND	180,000
	(2)	ND	17,800
	(16)	ND	34,600
<b>5</b>		ND	500,000
	(2)	ND	26,900
	(16)	ND	89,700
<b>6</b>		ND	87,600
	(2)	ND	13,350
	(16)	ND	23,230
<b>7</b>		ND	255,000
	(2)	ND	25,000
	(16)	ND	100,000

8		ND	55,700
	(2)	ND	7,000
	(16)	ND	14,200
1+9 (~1:1)		ND	5,100
10		ND	440,000
11		ND	850,000
12		ND	404,000
13		1.6	196,000
14		1.7	293,000
15		ND	127,000
16		3.4	150,000
17		ND	845,000
18		0.78	127,400
19		ND	67,200
20		ND	52,140
21		ND	243,000
22		ND	124,000
23		ND	36,500
24		0.67	240,000
25		ND	16,000

[0212] In the above table, all data are for RVP serotype-14 unless otherwise noted in parentheses. All strains of human rhinovirus (HRV) were purchased from American Type Culture Collection (ATCC) except for serotype 14, which was produced from the infectious cDNA clone constructed and supplied to Applicants by Dr. Robert Rueckert at the Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin. The column designated INHIB represents the percent inhibition at 10 minute preincubation with 50 nM RVP prior to addition of substrate. The data in the column designated  $k_{obs/I}$  was measured from progress curves in enzyme start experiments. The designation ND indicates that a value was not determined for that compound.

#### **Antirhinoviral HI-HeLa Cell Culture Assay**

[0213] In the Cell Protection Assay, the ability of compounds to protect cells against HRV infection was measured by the XTT dye reduction method. This method is described in Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H., Boyd, M. R., *J. Natl. Cancer Inst.* **1989**, *81*, 577-586, the disclosure of which is incorporated herein by reference.

[0214] HI-HeLa cells were infected with HRV-14 at a multiplicity of infection (m.o.i.) of 0.13 (virus particles/cell) or mock-infected with medium only. Infected or mock-infected cells were resuspended at  $8 \times 10^5$  cells per mL and incubated with appropriate concentrations of compounds of formula I. Two days later, XTT/PMS was added to test plates, and the amount of formazan produced was quantified spectrophotometrically at 450/650 nm. The  $EC_{50}$  was calculated as the concentration of compound that increased the percentage of formazan production in compound-treated, virus infected cells to 50% of that produced by compound-free mock-infected cells. The 50% cytotoxic dose ( $CC_{50}$ ) was calculated as the concentration of compound that decreased the percentage of formazan produced in compound-treated, mock-infected cells to 50% of that produced by compound-free mock-infected cells. The therapeutic index (TI) was calculated by dividing the  $CC_{50}$  by the  $EC_{50}$ .

[0215] All strains of human rhinovirus (HRV) for use in this assay were purchased from American Type Culture Collection (ATCC) except for HRV serotype-14, which was produced from the infectious cDNA clone constructed and supplied to Applicants by Dr. Robert Rueckert at the Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin. HRV stocks were propagated, and viral assays were performed in HI-HeLa cells (ATCC). Cells were grown in Minimal Essential Medium, available from Life Technologies, with 10% fetal bovine serum.

[0216] The compounds were tested against control compounds WIN 51711, WIN 52084, and WIN 54954, all obtained from Sterling-Winthrop Pharmaceuticals, and control compound Pirodavir, obtained from Janssen Pharmaceuticals. Antiviral data obtained for the test compounds are shown in Table 2 where all data are for HRV serotype-14 unless otherwise noted in parentheses.

**TABLE 2**

#	HRV	$EC_{50}$	$CC_{50}$	TI
---	-----	-----------	-----------	----

		( $\mu$ M)	( $\mu$ M)	
<b>1</b>		0.36	>320	>889
<b>2</b>		0.24	>320	>1333
	(2)	1.8	>320	>178
<b>3</b>		1.9	50.1	26
<b>4</b>		0.10	>320	>3200
	(2)	0.50	>320	>640
<b>5</b>		0.19	>320	>1730
<b>6</b>		0.68	>100	>147
<b>7</b>		0.022	>10	>454
	(2)	0.10	>10	>100
	(10)	0.035	>10	>286
	(89)	0.004	>10	>2500
	(39)	0.13	>10	>75
<b>8</b>		0.19	>100	>526
<b>1+9</b> (~1:1)		1.3	>320	>246
	(16)	2.8	>320	>114
	(2)	2.0	>320	>160
	(10)	4.1	>320	>78
	(89)	5.1	>320	>63
<b>10</b>		0.011	>1	>91
	(2)	0.18	>1	>57



<b>11</b>		0.006	>3	>500
	(2)	0.12	>3	>25
	(39)	0.13	>3	>23
	(10)	0.060	>3	>50
	(16)	0.025	>3	>120
	(1A)	0.16	>3	>18
<b>12</b>		0.14	>1	>7
<b>13</b>		0.060	>3	>50
<b>14</b>		0.020	>10	>500
	(2)	0.13	>10	>76
	(89)	0.080	>10	>125
	(16)	0.10	>10	>100
	(10)	0.16	>10	>62
	(1A)	0.17	>10	>58
	(39)	0.07	>10	>143
<b>15</b>		0.063	>1	>15
<b>16</b>		0.050	>3	>60
	(2)	0.18	>3	>16
	(39)	0.20	>3	>15
	(89)	0.080	>3	>37
	(16)	0.10	>3	>30
	(10)	0.15	>3	>20

	(1A)	0.18	>3	>16
<b>17</b>		0.027	>10	>370
	(2)	0.36	>10	>27
	(39)	0.48	>10	>20
<b>18</b>		0.48	>3	>6
<b>19</b>		0.28	>3	>10
	(2)	0.71	>3	>4
	(10)	1.6	>3	>1.8
	(1A)	0.60	>3	>5
<b>20</b>		0.042	>3	>71
	(2)	0.56	>3	>5
	(39)	1.2	>3	>2.5
	(89)	0.47	>3	>6
	(16)	0.15	>3	>20
	(10)	0.50	>3	>6
	(1A)	0.53	>3	>5
<b>21</b>		0.16	>10	>62
	(2)	1.0	>10	>10
	(10)	1.4	>10	>7
	(1A)	0.56	>10	>17
<b>22</b>		0.050	>32	>640
	(2)	0.36	>32	>88

	(39)	0.40	>32	>80
	(89)	0.24	>32	>133
	(16)	0.40	>32	>80
	(10)	0.50	>32	>64
	(1A)	0.43	>32	>74
<b>23</b>		20.9	>10	ND
<b>24</b>		0.032	>3	>93
<b>25</b>		0.14	>3	>21
	(2)	0.68	>3	>4
	(10)	1.7	>3	>1.7
	(1A)	0.90	>3	>3
	(16)	0.71	>3	>4
	(39)	0.56	>3	>5
	(89)	0.32	>3	>9
<b>WIN 51711</b>		0.78	>60	>77
<b>WIN 52084</b>		0.07	>10	>143
<b>WIN 54954</b>		2.13	>63	>30
<b>Piro- davir</b>		0.03	>10	>300

#### **Anticoxsackieviral HI-HeLa Cell Culture Assay**

[0217] The Coxsackie strain A-21 (CVA-21) was purchased from American Type Culture Collection (ATCC). Virus stocks were propagated, and antiviral assays

were performed in HI-HeLa cells (ATCC). Cells were grown in Minimal Essential Medium with 10% fetal bovine serum.

**[0218]** The ability of compounds to protect cells against CVA-21 infection was measured by the XTT dye reduction method. This method is described in Weislow, O. S., Kiser, R., Fine, D. L., Bader, J., Shoemaker, R. H., Boyd, M. R., *J. Natl. Cancer Inst.* **1989**, *81*, 577-586, the disclosure of which is incorporated herein by reference. HI-HeLa cells were infected with CVA-21 at a multiplicity of infection (m.o.i.) of 0.05 (CVA-21) or mock-infected with medium only. Infected or uninfected cells were resuspended at  $4 \times 10^4$  cells per mL and incubated with appropriate concentrations of drug. One day later, XTT/PMS was added to test plates, and the amount of formazan produced was quantified spectrophotometrically at 450/650 nm. The  $EC_{50}$  was calculated as the concentration of drug that increased the percentage of formazan production in drug-treated, virus-infected cells to 50% of that produced by drug-free, uninfected cells. The 50% cytotoxic dose ( $CC_{50}$ ) was calculated as the concentration of drug that decreased the percentage of formazan produced in drug-treated, uninfected cells to 50% of that produced in drug-free, uninfected cells. The therapeutic index (TI) was calculated by dividing the  $CC_{50}$  by the  $EC_{50}$ .

**[0219]** The compounds were tested against control compound WIN 54954, obtained from Sterling-Winthrop Pharmaceuticals, and control compound Pirodavir, obtained from Janssen Pharmaceuticals. Antiviral data obtained for the test compounds against Coxsackie strain A-21 (CVA-21) are shown in Table 3.

Table 3

COMPOUND #	$EC_{50}$ ( $\mu$ M)	$CC_{50}$ ( $\mu$ M)	TI
<b>7</b>	0.16	>10	>63
<b>WIN 54954</b>	>100	>100	

<b>Pirodavir</b>	>100	>100	
------------------	------	------	--

[0220] In describing the invention, the inventors have set forth certain theories and mechanisms in an effort to disclose how or why the invention works in the manner in which it works. These theories and mechanisms are set forth for informational purposes only. Applicants are not to be bound by any specific chemical or physical mechanisms or theories of operation.

[0221] While the invention has been described in terms of various preferred embodiments and specific examples, those skilled in the art will recognize that various changes and modifications can be made without departing from the spirit and scope of the invention, as defined in the appended claims.